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GASTRIC ANALYSIS*

THE SECRETION CURVE AS DEVELOPED BY FRACTIONAL ANALYSIS

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THE use of the stomach tube for diagnostic examination of the stomach contents received an impetus by the introduction and use of the gastroduodenal tube for securing the gastric contents before, and fractionally during the digestive cycle. The method has been extensively used during the past ten years and large contributions made to the study of the gastric function by Gros, Skaller, Palifaski, Bert, Fishbaugh, Hawk, and Rehfuss. The greater effectiveness of the small tube as compared to the large caliber tube in emptying the stomach and the facility of its clinical use are generally conceded. Recently, a number of writers have criticised the fractional method of gastric analysis. Wheelon,[1] Gorham,[2] Kopeloff[3] and White[4] have published articles to show that fractions of the stomach contents withdrawn periodically are not aliquot portions, and the method has on that premise been decried as not giving a dependable curve of the acidities during the digestive cycle. The practice of gastric analysis has fallen into greater disuse because of this. The improving and increasing use of the x-ray and its more direct visual approach in the examination of the stomach has also done much to discourage the practice of gastric analysis. The two methods should be used as supplementary.

Kopeloff claims that single determinations of gastric acidity by the Rehfuss method are not sufficient on which to base conclusions, because they do not take into consideration individual variation, and that the aliquot fractions are not representative. White concludes that only gross changes in acidity have clinical importance. He found that, without mixing, the av-

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erage variation of free HCl was 17 and of the total acid was 22, and that, with mixing, the average variation of free HCl was 10 and of the total acid was 8.5.

Wheelon suggests the following causes for the variations which he found in the samples: Shifting of the tube tip changes portion of the stomach from which the tube delivers; rapid emptying of the stomach alters degree of acid concentration; response of given stomach to a test meal may not be usual; sudden withdrawal may have an effect; and regurgitation of the duodenal contents may alter the gastric acidities. These factors can operate to disturb the validity of the samples as representative portions of the gastric contents; but, in the experience recorded in this paper they have not so operated with any disturbing frequency.

The procedure of the gastric analyses forming the material of this paper is as follows: The Rehfuss gastroduodenal tube is used. The patient is fasting and without fluid from the previous evening. The fasting gastric con-

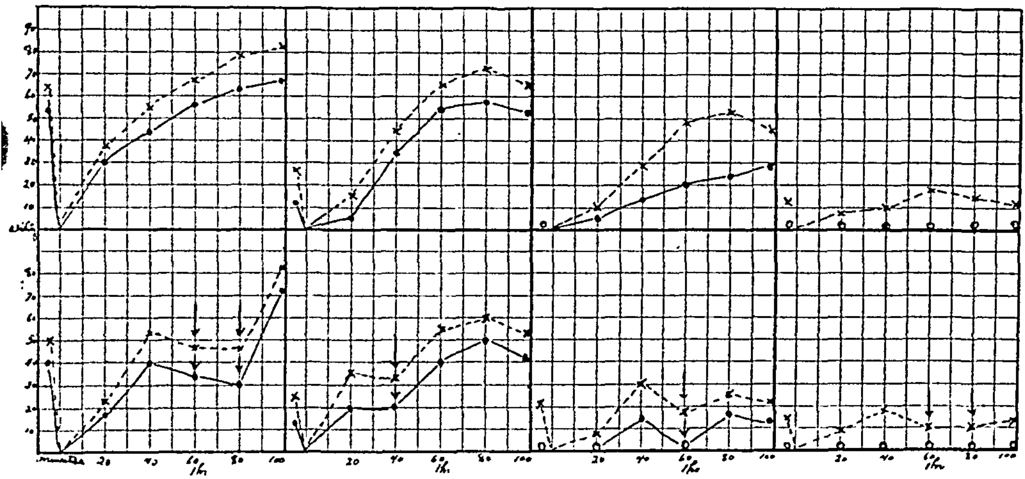

Fig. 1.—Selected Actual Curves.

Upper row shows curves with all samples valid.
Lower row shows curves with invalid titrations indicated by arrow. (See text.)

tents are aspirated, and the test meal is then taken. The test meal consists of one shredded wheat biscuit (ground) and 400 c.c. of water (two tumblers), with 1 c.c. of phenolsulphonephthalein. Aspirations of 10 c.c. samples are made every twenty minutes for five samples and with the fifth the stomach is emptied. Before and after each aspiration one syringe full of air is injected. The fasting contents are measured, examined, and titrated. Each sample is titrated and examined. The last sample is measured.

Two hundred and fifty-seven cases were subjected to 275 analyses. This experience shows that the curves of the gastric secretion as revealed by fractional analysis show an ascending gradient followed by a descending gradient and that accidental variations of the fractions aspirated are not of a degree or frequency as to vitiate these curves. Among these 2487 titrations of 1244 samples in 275 curves, there appears a contrary variation from this usual gradient, by one sample in 123 curves, and by two samples in 29 curves, and

in only 9.1 per cent of the titrations. Such conformation to the curve gradient would seem to contradict the results of White, Wheelon, and Gorham.

Fractional gastric analysis as practiced does not reveal the physiology of the stomach in all its separate phases and does not exhibit these in an absolute evaluation, but rather the curve of the fractional analysis is the composite resultant of the present (occasional) factors in the physiologic status of the stomach, as modified by the artificial standardized factors introduced (dilution, tube presence, nonpalatable meal, aspiration and air injection) into the test.

Samples are considered valid if their titration value follows the direction of the curve in its normal ascent and descent. A sample is considered invalid if the titration value fails to follow the curve direction, by being equal to the titration immediately preceding, or if it reverses the gradient of the curve. Persistence of the zero of achlorhyrdia is not considered as invalidating the samples for HCl. Then the titration of the total acid only shows discrepancies. Samples are invalidated for failure either in the curve of free HCl or in that of total acid. A high titration in the first sample is considered as a failure to aspirate entirely the fasting contents and is always associated with a higher free acid in the fasting contents. Table I summarizes the percentage incidence of invalid samples.

Table I

Summary

	Total	
Cases	257	
Curves	275	
Titrations of fractional samples	2487	
Titrations falling into invalid position on curves	227	9.1%
Curves having one or more invalid samples	123	44.8%
Curves having two invalid samples	29	10.5%

Table II

Type of Curves

	Total	Hyperacidity	Fasting Hyperacidity Normal	Normal	Hypoacidity	Achlorhydria	Male	Female
Total	257	42	23	89	71	32	115	142
Duodenal Ulcer	45	28	4	10	2	1	39	6
Gastric Ulcer	6	1	–	2	2	1	–	6
Gastric Cancer	2	–	–	–	–	2	2	–
Gastric Syphilis	1	–	–	1	–	–	1	–
Gall Stones	12	–	1	5	5	1	2	10
Gall bladder Disease	25	–	1	9	12	3	4	21
Appendicitis, Chronic	23	1	1	9	7	5	9	14
Gastritis, Chronic	7	2	–	3	–	2	4	3
Gastric Motor Delay, functional	3	–	–	3	–	–	2	1
Dyspepsia Nervosa	28	2	4	11	10	1	17	11
Migraine	5	–	1	1	3	–	–	5
Enteritis, Acute	5	–	1	1	3	–	–	5
Constipation, Chronic	25	2	2	13	8	–	9	16
Colitis	11	1	1	5	2	2	4	7
Tuberculosis, Pulmonary	4	–	–	2	1	1	3	1
Anemia, Simple	1	–	–	–	–	1	–	1
Neurasthenia	11	1	2	5	1	2	4	7
Autonomic Imbalance	7	–	–	1	2	4	3	4
Cirrhosis of the Liver	4	1	1	–	1	1	3	1
Other Diagnoses	32	3	4	8	12	5	9	23

In tabulating these curves to show the relation of the secretory activity to various conditions, they have been classified according to the highest hydrochloric acid concentration reached during the period. Hyperacidity indicates a value of HCl greater than 50 and does not mean the secretion of an acid of an abnormally high concentration. Normal acidity indicates values between 30 and 50. Hypoacidity indicates positive values under 30, and achlorhydria indicates the continued absence of free HCl.

Curves showing a hyperacidity in the fasting contents with a secretory curve within normal limits were made a separate group. Hyperacidity curves were of significant greater frequency in duodenal ulcer; gall bladder and appendix syndromes show a definite tendency to hypoacidities.

In the twelve cases having two or more gastric analyses there was a correspondence in the repeated curves, or a variation consistent with the clinical and x-ray findings in the cases. Two cases of duodenal ulcer showed, in two and three curves respectively, an increase in the acidities corresponding to the improvement of gastric motility as shown by x-ray. One case of acute gastroenteritis showed an increase in acidities of the second curve corresponding to the clinical and x-ray improvement. One case showing an achlorhydria initially showed a hypoacidity in an early repeated curve which was made because of a suggested psychic inhibition of secretion. This small experience in repeated curves has shown either a lack of variation or a logical correspondence to the clinical progress.

DISCUSSION

This analysis of a clinical experience with fractional gastric analysis gives an assurance in its validity for demonstrating the physiologic response of the stomach to the standardized factors of the method. The valuable data of gastric secretion have not been better developed than by this method. It does demonstrate alterations in gastric secretion and motility which are more or less directly diagnostic and helpful therapeutically. The demonstration of an achlorhydria is prompt and conclusive by this method. The extent to which the data developed by this method of gastric analysis may be diagnostically or therapeutically useful is more limited than has been claimed for practical deductions from analogous data in the past.

Gastric analysis cannot be discarded as being altogether superceded or replaced by newer methods of gastrointestinal examination. The examination of the fasting contents of the stomach is essential to gastrointestinal diagnosis, and the observation of the curve of the gastric acidities by the slow fractional method may give useful and necessary information. Knowledge of the gastric acidities has been of the greatest help in selecting a diet especially with consideration of the buffer values of various foods.

White asks if the practice of fractional gastric analysis is worth its time and effort. There is a confusing multiplicity of present clinical diagnostic procedures. Time alone, not to mention the wear and tear upon a patient, demands a thoughtful selection. Observation of the fasting gastric contents and the demonstration of active or absent hydrochloric acid secretion are essential. The demonstration of an achlorhydria has high clinical value in

other than gastrointestinal conditions. It occurred in 12.2 per cent of this series. Vanderhoof,[5] in a series of 4281 patients subjected to gastric analysis, shows an incidence of achylia gastrica of 10.5 per cent. He emphasizes that "there is good reason to believe that every person with true achylia gastrica has a potential case of either pernicious anemia or combined spinal sclerosis." The demonstration of hydrochloric acid in the fasting contents may obviate the need for developing the secretory curve where the x-ray is used for demonstrating gastric motility and organic changes.

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THE LEVINSON TEST AND OTHER LABORATORY STUDIES IN TUBERCULOUS MENINGITIS*

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THE typical cerebrospinal fluid in tuberculous meningitis is generally clear or slightly turbid, increased in amount, and under increased pressure. The globulin is increased, there is a moderate increase in the leucocytes with the small lymphocyte greatly predominating, and the sugar content is within normal limits. These findings do not per se confirm the diagnosis of tuberculous meningitis, nor do they exclude other possibilities such as cerebrospinal lues, paresis, encephalitis, poliomyelitis, etc. Further than this, cases of tuberculous meningitis have been reported in which the predominating cell was the polymorphonuclear leucocyte; hence in these rare cases we meet the possibility of confusing the condition with a purulent meningitis, providing the bacteriologic examination is negative, as sometimes happens, especially in epidemic meningitis.

We have various tests that help in differentiating some of the conditions resembling tuberculous meningitis, the Wassermann reaction and the colloidal gold would almost rule out lues, a high sugar content will usually exclude encephalitis, etc. But these are time consuming and difficult except for the experienced worker and often not available.

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The crucial test in proving that the fluid in question is tuberculous is, of course, the finding of the acid-fast organism. This is not an easy task and frequently unsuccessful and although, as a last resort a guinea pig inoculation can be made, the report is necessaarily delayed five to six weeks.

Various procedures have been suggested for finding tubercle bacilli. Allowing the fluid to stand undisturbed overnight in an incubator is supposed by some to increase the number of organisms, and others advise the addition of an equal volume of 95 per cent alcohol when there is no pellicle. While the pellicle is often present in tuberculous spinal fluids, many have the notion that it is only found in tuberculosis. This impression we want to correct, as we have seen pellicles in other conditions. When, however, a pellicle does form, it enhances our chances of finding the bacillus.

Of the various chemical tests that have been suggested, we have found the precipitation test of Levinson[1] extremely valuable. Proteins are precipitated as albuminates when treated with certain metallic salts, such as copper sulphate, zinc chloride, bichloride of mercury and so forth, and precipitated as insoluble salts when treated with weak organic acids, such as certain of the alkaloidal reagents, picric, phosphotungstic, tannic, and sulphosalicylic acids.

Levinson claims that in cases of suppurative meningitis the sulphosalicylic acid throws down a precipitate, the height of which in millimeters is three times as great as that thrown down by the mercuric chloride, whereas in tuberculous meningitis the reverse is the case, i. e., the bichloride precipitate is twice that of the sulphosalicylic acid.

While the protein content of the fluid in cerebrospinal meningitis is usually greater than in tuberculous meningitis, the range according to Mestrezat[2] being from 0.15 to 0.85 per cent for epidemic meningitis and from 0.13 to 0.56 per cent for tuberculous, Tashiro and Levinson[3] concluded, after carefully conducted experiments, that the test did not depend on the relative amounts of protein present, but on the hydrogen-ion concentration.

The changes in the hydrogen-ion concentration of the fluids on standing was investigated and a difference was noted, which was in itself sufficient to alter the electrical charges of the proteins. They found in epidemic meningitis that most of the proteins are positively charged, while in tuberculous meningitis the negatively charged proteins predominate. Hence, the more positively charged proteins present in epidemic meningitis give a higher precipitate with the sulphosalicylic acid which is negatively charged, than with the bichloride of mercury which is positively charged, the reverse being the case in tuberculous spinal fluid. This phase of the problem was not investigated by us.

We have used various alkaloidal metallic precipitants, but the results at best equal those obtained with the two reagents proposed by Levinson. We have used various dilutions of the spinal fluid, and various strengths of the reagents. In very cloudy spinal fluids it might be advisable to use various dilutions of the spinal fluid. The merits of the stronger solutions, along with the original test as proposed by Levinson will be given at length as we describe our work.

A study of tuberculous meningitis was undertaken with this test in view. We found that in a number of cases in which we were able to establish the presence of the tubercle bacilli in the spinal fluid, either before death or at autopsy, the one to two ratio of the two precipitates held good in practically all of our cases, furthermore, that in most of the cases we got this one to two ratio before finding the bacillus.

The following routine was observed: The spinal fluid was received in two sterile test tubes, one being used for cell count, differential, and precipitation test. In doing this test at first, we followed Levinson's technic. Small test tubes of about 8 mm. in diameter were used We placed one cubic centimeter of spinal fluid in each of two tubes. To one we added one cubic centimeter of a 1 per cent mercuric chloride and to the second one cubic centimeter of a 3 per cent sulphosalicylic acid. The tubes were shaken well, stoppered and stood at room temperature for twenty-four hours. At the end

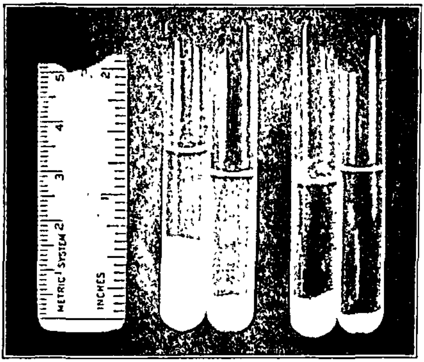

Fig. 1.—Right to left: 1. Mercuric chloride; 2, sulphosalicylic acid, three days before death. Ratio 1:3+. T. B. found; 3, mercuric chloride; 4, sulphosalicylic acid, one week before death. Ratio 1:2+. No T B. found.

of that time the column of precipitate was measured in millimeters. The second tube of spinal fluid we left undisturbed, until a pellicle had formed. This usually took place in less than twelve to fourteen hours. The pellicle was then fished out, teased out well, and stained for tubercle bacilli in the routine manner. When no pellicle was formed, we centrifuged at high speed for one-half hour. This sometimes forms a small pellicle, or else by the addition of 95 per cent alcohol, we precipitated the proteins and on centrifuging a compact sediment results. Occasionally, we had to resort to a guinea pig inoculation.

The two precipitates are of an entirely different character, that of the sulphosalicylic acid is heavy and compact and starts to form immediately, while that of the mercuric chloride is light, feathery, and forms slowly. Sometimes the precipitate does not come down into a compact sediment. It seems as if little floccules become adherent to the walls of the test tube. We

found it generally advisable to shake the tubes two to three hours before making the final reading. If one bears in mind that it is not the amount of protein thrown down in the two precipitates but the relative height of the column of protein in millimeters in each tube, the results are more uniform.

Another difficulty encountered was that in some cases we did not obtain a precipitate, but the tube remained cloudy. It was thought that in these cases either lower dilutions of the fluid or stronger reagents would avoid this difficulty. Better results were obtained by the use of stronger reagents, and since then we use 1 per cent and 2 per cent bichloride and 3 per cent and 6 per cent sulphosalicylic acid solutions.

With reference to the use of these higher strengths an analogy with the use of the cholesterin antigen in the Wassermann reaction seems permissible. The use of the cholesterin antigen alone would no doubt increase the number of false positive reactions, as well as the strength of the positive test, yet no serologist at present would do without it, using a less sensitive antigen as well. The same applies to the use of the stronger solutions in this test. It is valuable in borderline cases and especially where the lower solutions fail to bring down a sediment.

The results of our investigations are given in Tables I, II, III, and IV, but only those atypical or interesting cases will be given in detail.

J. R., an adult male was admitted to the Psychopathic Wards of the Philadelphia General Hospital. The provisional diagnosis was lethargic encephalitis. Study of the cerebrospinal fluid showed 217 cells per cu. mm. of which 4 per cent were polymorphonuclears and 96 per cent lymphocytes. No organisms were found on smear or culture. The Wassermann and the colloidal gold excluded syphilis of the nervous system. The Levinson test gave a precipitate of 3 mm. in the sulphosalicylic acid and 9 mm. in the bichloride, a ratio of 1:3. Because of this, a guinea pig was injected and five weeks later the animal was autopsied. Typical lesions were found and tubercle bacilli recovered. The patient was removed to the Jewish Hospital of Philadelphia, and the organism was found in the spinal fluid. This case illustrates the diagnostic value of the test. It is unfortunate that we have not obtained any fluids from cases of lethargic encephalitis.

J. L., an adult male was admitted to the Men's Tuberculous Wards of the Philadelphia General Hospital with definite symptoms of pulmonary tuberculosis. Later the patient developed symptoms and signs of meningitis. On the first lumbar puncture fluid the Levinson test was indicative of tuberculous meningitis, but no organisms were found. Three days later, another puncture was done, the Levinson ratio was 1:3.4, and this time we were successful in finding the tubercle bacilli.

This case illustrates a not uncommon experience, namely, that the Levinson test is generally positive before the organism can be found.

R. W., an adult male. This patient was admitted to the Medical Wards of the Philadelphia General Hospital with tuberculous cervical adenitis and symptoms and signs suggestive of meningitis. This case is reported because of the interesting cytology.

COUNT	CELL COUNT	POLYS PER CENT	LYMPHS PER CENT	RATIO OLD SOL.	RATIO NEW SOL.	T. B.
1	1350	75	25	1:2.5	1:2.9	–
2	56*	29	69	1:1.75	1:2.5	+
3	430	25	75	1:2.5	1:2.8	–

*Pellicle present.

TABLE I

LEVINSON TEST AND SPINAL FLUID FINDINGS IN TUBERCULOUS MENINGITIS

CASE	HEIGHT IN MM.		RATIO	CELL COUNT	DIFFERENTIAL			CEREBROSPINAL FLUID		T. B. IN SPINAL FLUID	AUTOPSY FINDINGS (BRAIN)
	Sul. 3 Per Cent	$HgCl_2$ 1 Per Cent			Polys. Per Cent	Endo. Per Cent	Lymphos. Per Cent	Wassermann	Colloidal Gold		
J.L.	5	17	1: 3.4	130	3	1	96	–	0011123210	–	0
				183	10	9	81			+	
T.H.	10	22	1: 2.2	156	–	–	100	–	2534444443	–	P.M.
											Military
											T.B.
C.H.	4	9	1: 2.3	75	–	–	100	–	None Made	–	
C.H.	5	13	1: 2.6	21	–	–	100	–		–	0
										P.M.L.P.	
										+	
J.B.	8	34	1: 4.2	25	–	–	Predom	–	None Made	+	0
							inate				
J.S.	4	8	1: 2.0	371	15	–	85	–	1245555433	–	
J.S.	4	11	1: 2.8	–	–	–	–			–	Yes
										P.M.B.S.+	+
E.G.	2	33	1:16.5	255	46	8	46	–	3455555320	–	
E.G.	4	32	1: 8.0	64	2	10	88			–	Yes
										P.M.B.S.+	+
W.Y.	3	5	1: 1.7	235	1	11	88	–	2445555511	–	
W.Y.	4	9	1: 2.3	182	2	8	90			+	Yes
											+
J.C.	5	20	1: 4.0	170	24	7	69	–	0013233222	–	
J.C.	–	–	–	316	8	3	79			+	Yes
											+
W.O.	6	20	1: 3.3	160	1	–	99	–	0001233311	–	
W.O.	6	18	1: 3.0	182	8	2	90			–	Yes
											+
J.R.	3	9	1: 3.0	217	4	18	78	–	0012221000	–	0
										G.P.+	
A.C.	2	4	1: 2.0	42	8	9	83	–	1233422100	–	Yes
											+

– Negative, or none found.
0 Not made.
P.M. Postmortem.
P.M.L.P.—Postmortem Lumbar Puncture.
P.M.B.S.—Postmortem Brain Smear.
G.P.—Guinea Pig Inoculation.

TABLE II

OLD AND NEW LEVINSON TEST AND SPINAL FLUID FINDINGS IN TUBERCULOUS MENINGITIS

CASE	HEIGHT IN MM. Sul. 3 Per Cent	HEIGHT IN MM. Hg Cl_2 1 Per Cent	RATIO OLD	HEIGHT IN MM. Sul. 6 Per Cent	HEIGHT IN MM. Hg Cl_2 2 Per Cent	RATIO NEW	CELL COUNT	DIFFERENTIAL COUNT Polys. Per Cent	DIFFERENTIAL COUNT Endo. Per Cent	DIFFERENTIAL COUNT Lympho. Per Cent	CEREBROSPINAL FLUID Wass.	CEREBROSPINAL FLUID Colloidal Gold	TUB. BAC. IN SPINAL FLUID	P.M. FINDINGS BRAIN
H.M.	3	8	1:2.7	3	12	1:4	52	–	–	Majority	–	0000111100	–	Yes
	3	6	1:2	4	14	1:3.5	75	4	10	86			–	+
J.G.$_1$	5	11	1:2.2	6	21	1:3.5	153	2	10	88	–	None made	–	Yes
J.G.$_2$	5	10	1:2	7	16	1:2.3	180	4	4	92			–	+
R.H.	3.5	5	1:1.4	4	19	1:4.7	162	–	17	83	–	None made	+	0
W.O.	6	20	1:3	Insuff.	27	?	160	1	–	99	–	0001233311	–	Yes
														+
J.C.$_1$	2	Cloudy	?	2	6	1:3	Not Made	–	–	–	–	0001110000	–	Yes
J.C.$_2$	3	9	1:3	5	20	1:4	177	3	12	85			–	+
W.N.	3	Cloudy	?	3	9	1:3	115	6	3	91	–	2334432110	+	Yes
														+
C.O.	12	Cloudy	?	12	24	1:2	500	2	4	94	–	None made	–	0
													G.P.+	
R.G.	6	Cloudy	?	6.5	20	1:3	133	5	8	87	–	1111232200	–	0
													P.M.L.P.	
													+	
													–	
M.G.	4	Cloudy	?	5	16	1:3.2	40	10	–	90	–	0000133210	P.M.L.P.	Yes
W.R.$_1$	4	10	1:2.5	4.5	13	1:2.9	1350	75	–	25	–		+	+
W.R.$_2$	4	7	1:1.75	4	10	1:2.5	56	29	2	69	–	3555555321	–	0
W.R.$_3$	3	7.5	1:2.5	4	11	1:2.8	430	25	–	75			+	
													–	

TABLE III

LEVINSON TEST AND SPINAL FLUID FINDINGS IN CEREBROSPINAL SYPHILIS

CASE	HEIGHT IN MM. Sul. 3 Per Cent	HEIGHT IN MM. Hg Cl_2 1 Per Cent	RATIO	CELL COUNT	CEREBROSPINAL FLUID Wass.	CEREBROSPINAL FLUID Colloidal Gold
J.J.	2	4	1:2	–	++++	5555554200
L.R.	3.5	6.5	1:1.8	107	++	5555543100
L.R.	4	5	1:1.3	89	++++	5555554310
T.N.	4	5	1:1.3	94	++++	5555555554
W.C.	3.5	5	1:1.3	7	++++	5555553100
R.B.	5	5	1:1.0	20	++++	5555555544
N.B.	3.75	4	1:1.1	7	++	5555554310
S.G.	3	4.5	1:1.5	94	++++	5555555511
E.N.	3	5	1:1.7	40	++++	5555555422
D.U.	3	6	1:2			
	3	4	1:1.3	393	++++	5555553311
T.S.	2	5	1:2.5	275	++++	1122110000
M.S.	4	5	1:1.3	1000	++++	None made –

TABLE IV

OLD AND NEW TEST AND SPINAL FLUID FINDINGS IN CEREBROSPINAL SYPHILIS

CASE	HEIGHT IN MM. Sul. 3 Per Cent	HEIGHT IN MM. Hg Cl_2 1 Per Cent	RATIO OLD	HEIGHT IN MM. Sul. 6 Per Cent	HEIGHT IN MM. Hg Cl_2 2 Per Cent	RATIO NEW	CELL COUNT	CEREBROSPINAL FLUID Wassermann	CEREBROSPINAL FLUID Colloidal Gold
E.B.	5.5	6	1:1.0	5	7	1:1.4	100	++++	5555555554
H.H.	7.5	11	1:1.5	7	14	1:2.0	108	++++	5555555433
E.C.	2	4	1:2.0	2	5	1:2.5	22	++++	2444321000
L.O.	4	3.5	1:1.1	4	5	1:1.3	80	++++	None Made
E.S.	5	6	1:1.2	6	7	1:1.2	24	++++	5555555542
C.B.	3	4	1:1.3	4	6	1:1.5	286	++++	2223442000
F.W.	3.5	8	1:2.3	4	11	1:2.8	23	++++	2224321000
F.M.	4	9	1:2.5	4	14	1:3.5	30	++++	4555544211
J.S.	5	7	1:1.4	5	8	1:1.6	78	++++	5555555543
W.R.	3	3	1:1	3	5	1:1.7	118	++++	4444311110
J.F.	3	4	1:1.3	4	7	1:1.8	131	++++	4444431000
							1210		
H.C.	3	Cloudy	?	3	Cloudy	?	P 99	++++	2333442100
	4	Cloudy	?	2.5	2	1:3.1	L 1		

In this case the first spinal fluid showed 75 per cent polymorphonuclears and at this time the case might have been considered a purulent meningitis, yet the ratio, sulphosalicylic acid to bichloride, was 1 : 2.9, suggesting tuberculous meningitis. In the next count the polys were diminished to 25 per cent. We had another case, E. G., Table I, in which there were 46 per cent polymorphonuclears and 46 per cent lymphocytes. This predominance of polymorphonuclears sometimes happens in tuberculous meningitis.

DISCUSSION

Results obtained with the Levinson test were gratifying with one exception, in the twenty cases of tuberculous meningitis reported. The ratio was 1 : 2 or above.

Furthermore, by using this test, we were encouraged to double our efforts in searching for tubercle bacilli and since success in finding the organism is almost in direct proportion to the efforts of the examiner, the test, in this sense, is also valuable.

TABLE V

LEVINSON TEST IN MISCELLANEOUS CONDITIONS

DIAGNOSIS	HEIGHT IN MM.		RATIO	HEIGHT IN MM.		RATIO	CELL COUNT	DIFFERENTIAL			CEREBROSPINAL FLUID	
	Sul. 3 Per Cent	Hg Cl_2 1 Per Cent		Sul. 6 Per Cent	Hg Cl_2 2 Per Cent			Poly. Per Cent	Endo. Per Cent	Lympho. Per Cent	Wassermann	Colloidal Gold
J. B. Pneumococcus meningitis after serum given	24	19	1.26:1	–	–	–	75	95	–	5	0	0
W. A. Pneumococcus meningitis after serum given	30	19	1.57:1	–	–	–	8,300	99	1	–	0	0
M. T. Pneumococcus meningitis before serum given	15	11	1.36:1	–	–	–	3,800	93	5	2	0	0
J. H. Meningococcus meningitis after serum given	5	3	1.66:1	–	–	–	6,700	78	22	–	0	0
M. R. Staphylococcus meningitis before serum given	3	Cloudy	?	4	5	1:1.2	830	100	–	–	Neg.	0001122100
F. P. Staphylococcus meningitis. Serum given between 2 tests	3.5 5	Cloudy Cloudy	? ?	4 7	7 7	1:1.7 1:1	1,240 1,660	99 99	– –	1 1	0	0
F. B. Influenza meningitis before serum given	5	Cloudy	?	4	13	1:3.5	5,800	100	–	–	Neg.	1111122000
E. McL. Influenza meningitis. Serum given	4 5 7 5	Cloudy 11 Cloudy 7	? 1:2.2 ? 1:1.4	– – – –	– – – –	– – – –	3,180 30 340 10,000	100 – 73 95	– – 10 –	– – 17 5	0	0
J. C. Encephalitis	4	7	1:1.7	–	–	–	12	–	–	–	Neg.	000C000000
W. McQ. 1. Glioma. 2. Basal meningitis	4.5	5	1:1.1	–	–	–	1,200	47	–	53	Neg.	000C000000

RESUMÉ OF TABLES I, II, III AND IV

		LESS THAN 1:2 RATES	1:2	1:2 TO 1:2.5	ABOVE 1:2.5	TOTAL
OLD TEST	T. B.	1	1	4	11	17
	C. S. LUES	17	3	3	0	23
NEW TEST	T. B.	0	1	-	9	10
	C. S. LUES	8	1	-	3	12

The 1 : 2 ratio was often established before the finding of the organism, while in other cases, in which the organism was not found, we had to either *inject a guinea pig, or await postmortem findings for confirmation.*

In three cases in which the tentative clinical diagnosis was not tuberculous meningitis, because of the results obtained with the precipitation test, we either made smears or injected a guinea pig, and thus independent of knowledge of signs and symptoms, or of a request for a bacteriologic examination, we made a diagnosis of tuberculous meningitis.

Before we began to use the stronger solutions, several cases came to observation in which, because no precipitate was obtained in the 1 per cent bichloride, further investigations to prove the cases tuberculous meningitis were discontinued. These cases were, of course, not included in this study. We believe that, with the stronger solution, this difficulty will be avoided and results will be more uniform.

Unfortunately, the 1: 2 ratio is not pathognomonic of tuberculous meningitis, as 6 cases of cerebrospinal syphilis out of 24 studied (25 per cent) gave a ratio of 1 : 2 or over. If, however, we consider a ratio above 1 : 2 as proper for tuberculous meningitis, which, in the light of our work, seems permissible, the value of the precipitation test in differentiating between cerebrospinal syphilis and tuberculous meningitis is increased. Then, on the basis of our rather short series, one out of 10 cases would give a 1 : 2 ratio (10 per cent); but, obviously, in a study of 10 cases of cerebrospinal syphilis and 10 of tuberculous meningitis, we face only an error of 5 per cent.

No definite conclusions can be drawn as to the behavior of the test in the other conditions given in Table V, as the number of cases were not sufficient to warrant any. Practically always a higher precipitate was obtained in the sulphosalicylic acid than in the bichloride solution.

SUMMARY

We have confirmed Levinson's findings in that with his test the height of the precipitate in the bichloride of mercury solution is twice that of the sulphosalicylic precipitate.

A 1 : 2 ratio is very suggestive, but not pathognomonic of tuberculous meningitis.

The use of stronger reagents along with those proposed by Levinson is suggested as increasing the diagnostic value of the test.

The test should be performed in every case of meningitis, because (a) it is easy to do, (b) it is inexpensive, and (c) it gives valuable diagnostic information.

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STUDIES ON THE ACTION OF HISTAMINE ON HUMAN GASTRIC SECRETION*

BY LOUIS M. GOMPERTZ, M.D., NEW HAVEN, CONN., AND MARTIN G. VORHAUS, M.D., NEW YORK, N. Y.

HISTAMINE has been used for a long time in pharmacologic laboratories. It has been found in the human intestines by many observers, some of whom attach little or no importance to its presence, while others believe that a large variety of disorders are associated with this compound.

When histamine is injected intravenously into animals, a marked drop in blood pressure is the most prominent effect noted. In animals with a diminished adrenal supply, the lowering of the blood pressure is much more marked and death may ensue as a result of the action of histamine. The pressor action of epinephrin, pituitrin and similar drugs is inhibited by an intravenous injection of histamine.

When injected subcutaneously into animals, this substance produces a stimulation of gastric secretion, sweating, salivation, and congestion of the mucous membranes with little or no drop in blood pressure.

Histamine (Iminazolylethylamin) is an amin derived from the amino-acid histidin, a cleavage product of the proteins. The production of this substance is believed to be due to a decarboxylization of the amino-acid brought about by bacterial action. This theory is supported by the work of Mellanby and Twort and by Berthelot and Bertrand, who isolated organisms capable of converting histidin into histamine.

Ivy and McIlvain applied histamine locally to the intestines of dogs in order to determine the possible reflex action on gastric secretion. When a 1:1000 solution of histamine was applied to the duodenal mucosa for twenty to thirty minutes, a marked secretion of gastric juice occurred regularly and without fail. There was a latent period from ten to thirty minutes.

Because of the observed effect of histamine on the gastric secretion Carnot, Koskowski, and Libert conducted a study of the gastric secretory response to histamine on human subjects. Subcutaneous injections of hista-

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mine were given to fourteen individuals, and an increased amount of gastric secretion was observed.

Matheson and Ammon reproduced this work on a small group of normal subjects using ergamine acid phosphate which contains approximately one-third of the histamine base. This work was very carefully carried out and errors by psychical stimulation of secretion were eliminated as far as possible. The results practically substantiated the previous conclusions showing an increased flow of gastric juice without any untoward effects in man.

Early in the spring of 1923 we planned our experimental work with histamine. On the basis of earlier reports on normal subjects, we proceeded to repeat the previous investigations and to study the gastric response to

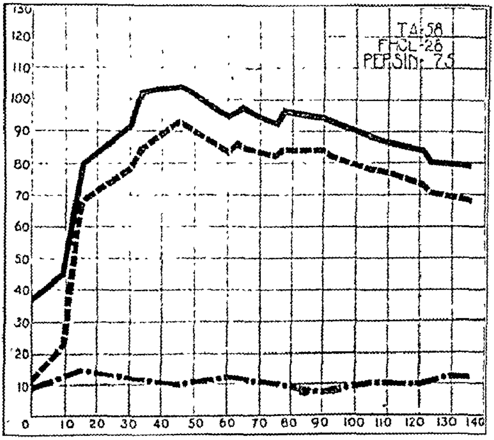

Fig. 1.—Showing a normal acidity after the Ewald test and a prolonged and increased response after the histamine test.
——— Total acidity; - - - - - - - - free hydrochloric acid; -.- -.-.-.- - pepsin (mm. digested).

histamine in various disorders of the stomach. We were interested in cases of achylia gastrica, in particular, because from a theoretical standpoint the reaction to this compound promised to offer a means of differentiating between true achylia, i.e., achylia with destruction of the secretory glands, and pseudo-achylia in which usually a functional inhibition of secretion occurs. In order to take into account the possible production of gastric secretion through irritation which might arise from the long continued presence of a tube within the stomach, the tube was allowed to remain in situ for one hour, the contents being removed at regular intervals before the injection of histamine. In no instance was any appreciable increase of secretion observed in this following manipulation.

In a number of patients saline solution was injected subcutaneously as

a control procedure and the stomach contents examined before administration of histamine. In all cases the effect of the saline injection was negative.

After satisfying ourselves that no increase of gastric secretion occurred by our method of investigation either from the stomach tube or injection per se, we proceeded as follows:

Forty-eight hours before the test an Ewald-Boas test meal was given to permit observations on the gastric phenomena, when no drug whatever was taken. No food or drink was given to the patients between supper the night before and the morning of the test. Early in the morning an Einhorn or Rehfuss tube was passed and the stomach contents were extracted with a Record syringe. In order to insure complete emptying of the organ the patient assumed different positions. After an interval of fifteen minutes the withdrawal of the fasting contents was repeated whereupon one c.c. of a 1:1000 solution of histamine hydrochloride was given subcutaneously. Thereafter at fifteen minute intervals the stomach was completely emptied of its contents, this procedure being continued until little or no secretion could be obtained. In some instances the tube was kept in the stomach for two and one-half hours or longer. At times we were compelled, because of the discomfort to the patient, to remove the tube before the secretion had ceased entirely. Occasionally fluid regurgitated through the tube before the end of the fifteen minute intervals. Such specimens were collected and recorded separately. Each sample was examined for volume, total acidity, "free" acidity, pepsin, blood, bile, and mucus. The pepsin content was estimated by the Mette method; the acidity was recorded in cubic centimeters of tenth-normal NaOH in acid.

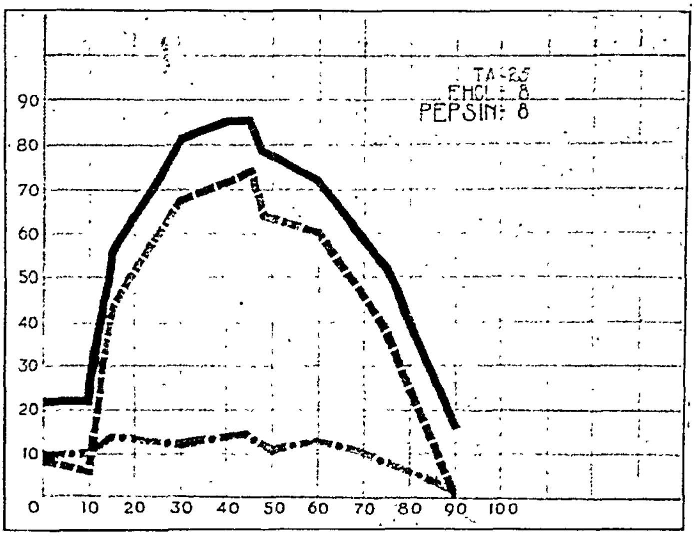

Fig. 2.—Showing a subacidity after the Ewald test and a normal secretory response of short duration after the histamine test.
——— Total acidity; -------- free hydrochloric acid; -.-.-.-.-.-.- pepsin (mm. digested).

The first twenty cases of our series were controlled with unusual care. During the course of the experiments, frequent blood pressure examinations

and pulse readings were made on all these persons. In many of them there was no demonstrable change after the subcutaneous injection of 1 c.c. of a 1:1000 solution of histamine; while in others there was a fall of only 6 to 8 mm. of mercury and an increase of 5 to 10 beats per minute in the pulse rate.

Up to the present date we have given over fifty injections of histamine without a disagreeable reaction. In the majority of the cases a flushing of the face and neck was noted. This came on in about five to ten minutes after the injection and lasted from fifteen to thirty minutes. Some of the patients complained of a mild headache and slight dizziness at about the same time, this latter symptom however being of a very transitory character.

The effects of subcutaneous injections of histamine have been interesting and in many instances quite surprising. In those persons in whom extraction after the Ewald meal revealed a normal or increased acidity, the re-

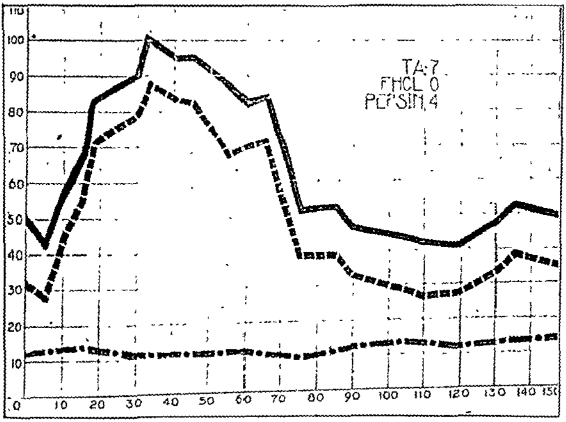

Fig. 3.—Showing an apparent anacidity after the Ewald test but the histamine response proves the secretory capacity to be within normal limits. The response is markedly prolonged. ———— Total acidity; -------- free hydrochloric acid; -.-.-.-.-.-.- pepsin (mm. digested).

sponse to histamine also was well marked. At times the acidity attained was very high, the total acidity reaching in one instance 200 and the "free" 180. In these cases the rate of secretion was considerably accelerated, one individual secreting 135 c.c. in fifteen minutes. In fact in one instance, almost 500 c.c. of secretion was obtained in two hours and the response had not entirely subsided at the end of that time. It was, however, in the patients with subacidity or anacidity after the Ewald meal that we observed the most surprising response to the histamine test. It was impossible to foretell the response to histamine from the results of the reaction to the test meal. In some, the acidity following the injection of histamine reached the same figures as in those patients who showed a normal or increased acidity after the Ewald meal.

Our first aim in these experiments was to substantiate the work already done. This we were prepared to do in the greater part. That histamine when given subcutaneously stimulates gastric secretion is a demonstrated fact. The injection of 1 c.c. of a 1:1000 solution of histamine hydrochloride appears to be a harmless procedure.

We were not able to substantiate the statement of Matheson and Ammon that histamine produces gastric reaction in definite sequences. They maintained that, after the injection of histamine in normal cases, the peptic activity reaches its maximum in about five to fifteen minutes; whereas the total and "free" acidities reach their maxima in about twenty to twenty-five minutes, the rate of secretion reaching its maximum last in from thirty to sixty minutes.

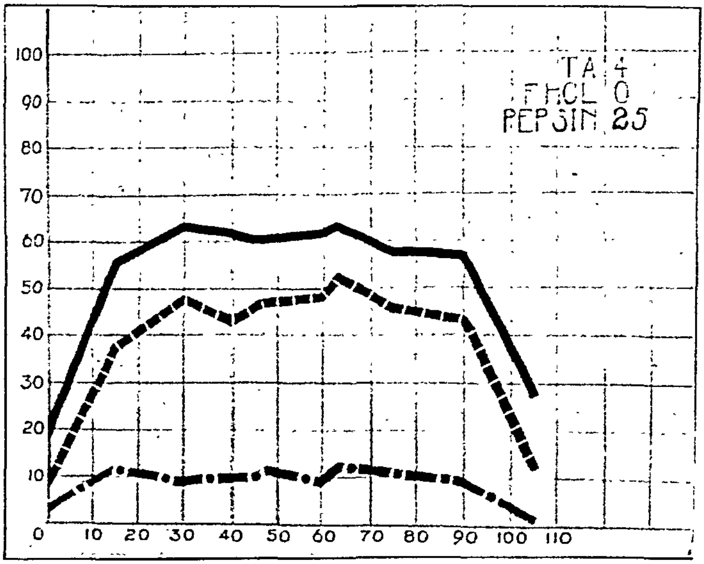

Fig. 4.—Showing an apparent anacidity after the Ewald test but the histamine response demonstrates a functional or pseudo-achylia. The secretory capacity is normal. ——— Total acidity; -------- free hydrochloric acid; –.–.–.–.–.– pepsin (mm. digested).

In our studies of abnormal cases, we were unable to determine any definite relationship between the increase in pepsin and acid production. In many patients the peptic activity reached its maximum after the acidity, in one instance the maximum of peptic activity being reached after the acidity had risen and fallen back to its fasting level.

Similarly, although 66 per cent of our cases showed their maximum rate of secretion in from thirty to sixty minutes after the injection, 22 per cent attained the maximum in from sixty to ninety minutes while 11 per cent reached it in fifteen to thirty minutes. Also in some cases the maximum of the rate of secretion was attained ten minutes before the maximum of acidity was secured. However, the differences between our results and those already reported are not very important and are perhaps explained by the fact that all our patients were the subjects of gastric disorders while the previous reports were based on studies of normal subjects.

By far the most interesting findings of our experimental studies were noted in the cases of apparent anacidity or so-called achylia gastrica. Because of this fact we shall describe our results in greater detail for this group. We included among them only such as showed a complete absence of "free" hydrochloric acid and a markedly diminished total acidity after the Ewald meal. Of seventeen cases in this group with an absence of "free" hydrochloric acid after the Ewald meal, ten or 59 per cent showed large amounts of "free" hydrochloric acid after the histamine test. The maximum amount of free acid production in these ten cases was as follows: 28, 32, 43, 46, 49, 50, 52, 57, 64 and 87 and the total acidity in each case was proportionately higher. In other words, of seventeen cases of apparent achylia gastrica, nine or more than 50 per cent produced normal or above normal acidities after the injection of histamine; while ten cases in all or

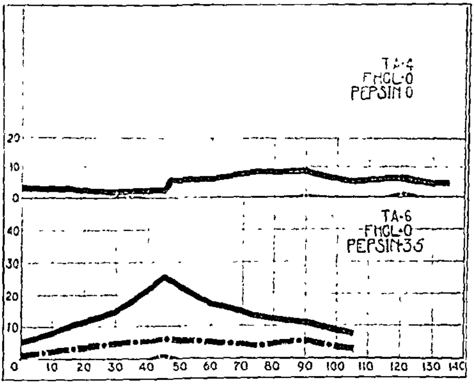

Fig. 5.—Above. Showing an anacidity after the Ewald test and proved by the absence of histamine response to be a case of achylia gastrica vera. *Below.* Same type of case as above except that there is a slight histamine response.
———Total acidity; - - - - - - - free hydrochloric acid, -.-.-.-.- pepsin (mm. digested).

59 per cent showed considerable amounts of free hydrochloric acid present. The diagnoses of these ten cases were as follows:

Alcoholic gastritis	2
Cholelithiasis	2
Chronic cholecystitis	1
Hydrops of gall bladder	1
Catarrhal jaundice	1
Carcinoma of the bile duct	1
Pulmonary tuberculosis with ptosis	1
Duodenal ulcer	1

It is therefore evident that of the seventeen assumed cases of achylia gastrica the histamine test excluded all ten, or 59 per cent. The diagnoses of the remaining seven follow:

Inoperable pyloric cancer	1
Ovarian cancer with pyloric and hepatic metastasis	1
Chronic gastritis and cholelithiasis	1
Achylia gastrica vera	4

The first three cases of this group did not at any time after the histamine injection show even a trace of free hydrochloric acid, the total acidity never rose above thirteen and pepsin was present in minimal amounts only. The last four, which we believe to represent true or organic achylia gastrica, were subjected to rigid examinations and repeated tests. These have at some time shown traces of free hydrochloric acid after histamine injections, never, however, exceeding 3 of free hydrochloric acid as a maximum figure. In one individual the total acidity rose from four to twenty-four following the histamine while the others also showed a rise in total acidity. Pepsin was present in almost normal amounts. We have followed these cases of achylia gastrica vera for several months, and improvement was noted on hydrochloric acid therapy. Repeated examinations have failed to show any demonstrable lesion of the stomach.

Thus by the use of the histamine test we were able to determine that of seventeen individuals with apparent achylia gastrica, ten were pseudo, functional or incomplete, while seven were organic. As a result of these experiments we feel that the histamine test is of distinct value in the differentiation between organic and functional achylia. At any rate we are not familiar with any other test which will permit equally satisfactory diagnosis of the capacity of the gastric glands to secrete free hydrochloric acid.

After reaching the conclusion that the histamine test offered a means of differential diagnosis in achylia gastrica, we centered our attention on its possible effect upon cases of early pyloric carcinoma. Our experience has as yet been too limited to permit final judgment regarding the possible value of the histamine test in this condition. In individuals suffering from carcinoma of the stomach the reaction has shown a very small increase, if any, over the Ewald figures. In no other gastric conditions investigated has the histamine response shown such a small increase over the Ewald figures. In view of these findings we believe that probably the carcinomatous patients were suffering from a disease which was destroying the secreting glands.

Whether, as Matheson and Ammon propose, histamine may be of use as a therapeutic agent in this field, remains to be demonstrated. Possibilities of its usefulness are suggested in cases of inanition due to tuberculosis, nervous shock or any condition where the discharge of hydrochloric acid and pepsin is inhibited. Histamine given subcutaneously immediately after a meal may promote secretion and consequently gastric digestion. Such indicated possibilities of usefulness deserve a clinical trial.

CONCLUSIONS

1. Histamine when injected subcutaneously into human subjects produces a stimulation of the flow of gastric secretion.

2. The subcutaneous injection of 1 c.c. of a 1:1000 solution of histamine hydrochloride is apparently harmless.

3. The histamine test is of value in the differentiation of true and pseudo achylia.

4. The histamine test *may* be of assistance in the early diagnosis of pyloric carcinoma.

5. Histamine offers possibilities as a therapeutic agent in conditions of diminished digestive function.

A COMPARISON OF THE INTRADERMAL AND SUBCUTANEOUS INJECTIONS OF INSULIN IN THE PRESENCE OF SUPRARENIN*

By E. F. Müller, M.D., and H. B. Corbitt, Ph.D., New York

THE action of insulin varies according to the tissues into which it is injected. This phenomenon has been discussed in previous publications[1, 2, 3] and the attempts made to explain it have given rise to many more questions related to the mechanism of the action of insulin.

It has been demonstrated that in normal rabbits the hypoglycemic action of a given quantity of insulin is dependent upon the route of administration. Intravenously, the action is early and of brief duration; given subcutaneously, the initial lowering occurs later and is more prolonged; given intradermally, the initial decrease approximates that observed after subcutaneous injection but its duration is much longer and the figures are lower both four and six hours after injection. Thus, it is shown that the further from the blood stream the insulin is injected, the more effective and prolonged is the influence exercised on the blood-sugar content. This was at first assumed to be due to a difference in the rate of absorption of the specific blood-sugar lowering substance. However, a study of the blood-sugar curves observed after different methods of administration, i.e., after injection into different body tissues reveals the fact that differences in the rate of absorption do not explain the resulting differences in the blood-sugar curves.

In order to obtain additional information which might be used in solving the problem of the mechanism of the action of certain drugs and its relation to the mode of administration, further studies were made in this field. These experiments involved a study of the action of insulin under the influence of simultaneous injections of suprarenin (synthetic epinephrin).

It is known that suprarenin injections increase the blood-sugar content but it should not be concluded that this action of suprarenin always tends to neutralize the action of insulin. On the contrary, as shown in a preliminary report,[4] suprarenin in some instances actually increases the action of insulin

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We have assumed from the results of other investigators,[2, 13, 14, 15, 16] that the caval pressure, if not increased, is at least effective even after massive doses of epinephrin.[12]

THE VAGI

In a majority of adult dogs, one cubic centimeter of 1 : 10,000 epinephrin solution injected intravenously produces (after the primary rise) an abrupt primary fall of blood pressure, which is due chiefly to central vagus stimulation. (See Figs. 1 and 2.) In some dogs, especially pups, epinephrin injections in doses from 1 to 30 c.c. failed to elicit a vagus stimulation or a primary fall, although the blood pressure rose unusually high. It is known that in very young animals the vagus is sometimes inactive.

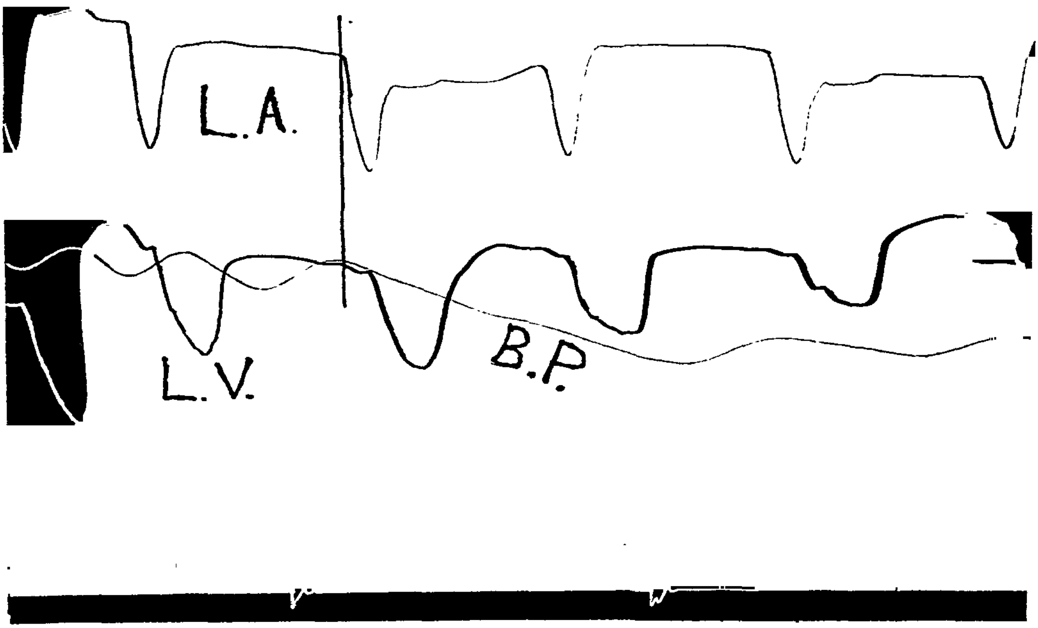


Fig. 1.—Left auricle and ventricle, systole=downstroke, spring levers. Time in seconds. Epinephrin 0.1 mg.: dog, 7 kilo. Peak of the primary rise and onset of the primary fall due to central vagus stimulation. For proof see text.

The proof that vagal stimulation is mainly responsible for a primary fall is based on the fact that after obtaining marked inhibition during the primary fall once or several times, section of the vagi in the neck, or 2 mg. atropine intravenously, change the entire contour of the blood pressure curve upon subsequent injections of the same dose of epinephrin, and in over 50 per cent of cases, eliminate the primary fall entirely. The blood pressure curve now describes a simple rise and fall.

The vagal action may be one of several types of sinus arrhythmia, as follows: (1) An inhibition of regular rate. (2) Coupled beats in which the weaker beat occurs after a longer pause; its volume ejection is less complete than the stronger beat which follows it after a shorter pause. The second beat appears to break through the vagal inhibition. (3) Phasic sinus arrhythmia of a more irregular character occurs more frequently from larger doses

of epinephrin (2 to 3 c.c.), but sometimes is quite distinct following a 1 c.c. injection. Sinus arrhythmia may produce several fluctuations following the primary rise, and in many cases a typical secondary rise does not occur. Numerous variations have been noted from simple predominating vagal inhibition to extreme fluctuations of vagal and accelerator activity. The larger doses tend to stimulate the accelerators sufficiently to break through the vagal influence more often, and as a result, several fluctuations occur. The mechanism of each succeeding rise and fall is usually identical with its primary rise and fall until blood pressure falls to normal or below.

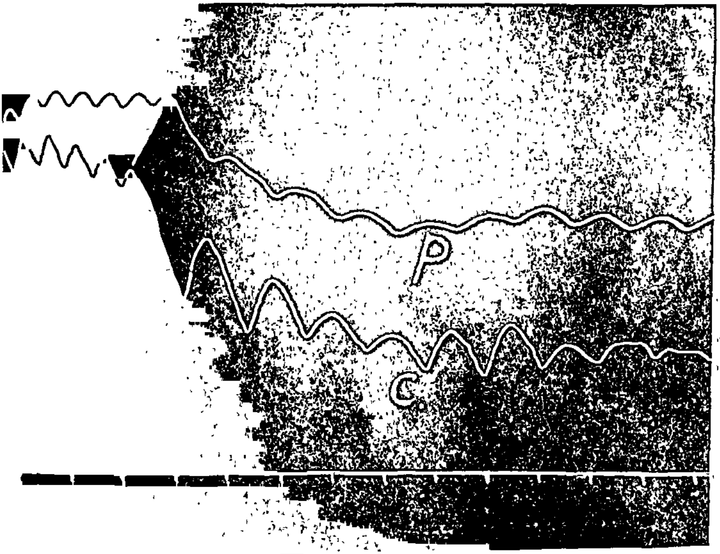


Fig. 2.—Pulmonary and carotid blood pressures. Time in 0.5 seconds. Peak of primary rise and onset of primary fall due chiefly to central vagus stimulation. For proof see test.

Vagal inhibition does not always cause a primary fall. This is evident if the slowing occurs early on the primary rise, if the onset is gradual, or if the inhibition is not marked. Apparently, in such cases the increased systolic discharge is sufficient to maintain the blood pressure.

The changes described hereafter occur also with the vagi severed.

ATRIOVENTRICULAR BLOCK

Atrioventricular block may occur after section of the vagi or atropine. It is usually of the irregular or 2-1 types, and in some cases is the cause of a primary fall. (See Fig. 3.) The onset of the block may or may not inaugurate the primary fall. In the latter case, the blood pressure begins to fall

for several beats preceding the block. The only constant change we have observed in the heart, preceding the block, is a decrease of the systolic ejection gradient. Incomplete block occurs more often if chloroform is administered. The "delayed inhibition" described by Oliver and Schafer[1] and the very marked inhibition following adrenalin (chloroform anesthesia) reported by Heinekamp[17] have been in my experiments not sequential inhibition, but incomplete atrioventricular block. The immediate effect of the block is a marked fall of pressure. (See Fig. 3.) As the systolic discharge increases, the pressure soon begins a slow rise, and with the return of normal sequence, becomes quite abrupt. The blood pressure picture varies with the duration of the block, which may be from a few seconds to a few moments.

The larger doses of epinephrin may produce an apparent disassociation of auricles and ventricles without bradycardia. The disassociation, or at least the auricular filling function, is often intermittent, and, likewise, produces

Fig. 3.—Right auricle and left ventricle, spring levers, systole=downstroke. Time in seconds. Dog, 9 kilo: vagi severed: epinephrin 0.15 mg. Peak of primary rise and onset of primary fall due to irregular atrioventricular block. A few beats later this became established as a 2-1 block, which continued for about thirty seconds.

fluctuations of blood pressure. The secondary rise occurs with the return of normal sequence. Kahn[18] reported complete heart block following epinephrin injection, without bradycardia. Epinephrin, it is said, also causes acceleration of both auricles and ventricles after experimental block.[19, 20, 21]

PREMATURE CONTRACTIONS

Premature contractions of ventricular origin may contribute to or cause a primary fall. They may occur as isolated contractures, bigeminy, trigeminy, or in a series which predominates the rhythm. The isolated premature contractions are common with ether anesthesia, whereas under chloroform anesthesia the complexes also frequently appear. Such irregularities have been described also by Levy and Lewis.[22, 23]

Premature auricular contractions often occur after epinephrin, but are not in themselves a specific cause for a primary fall of pressure.

VARIATIONS OF CONTRACTION PROCESS

A hypodynamic heart (chloroform or exposure) which responds to epinephrin, shows at first a decrease of volume from the cardiac acceleration. However, as the blood pressure rises, dilatation again becomes evident. A heart in good condition shows very little change in volume until the blood pressure rises considerably, at which time there may be a variable change in volume. A common occurrence at the peak of the primary rise is the development of an apparent depression of contractile power, which is a frequent cause for a primary fall. This temporary insufficiency may be manifested in several ways, as (1) pulsus alternans, (2) incomplete systole and diastole, (3) incomplete systolic effort with acute dilatation.

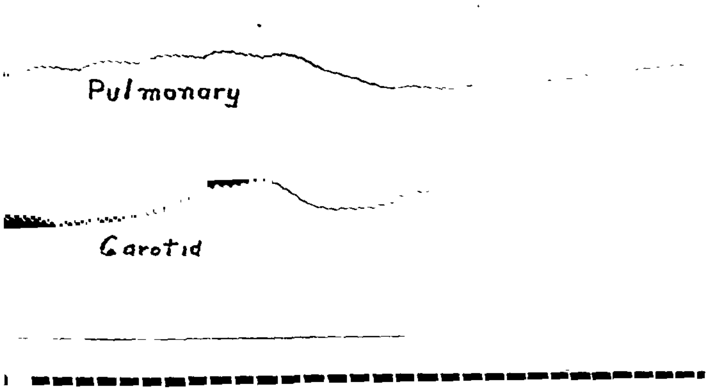


Fig. 4.—Pulmonary carotid blood pressures, time in seconds. Dog, 6 kilo; vagi cut. Primary rise and fall and secondary rise following 0.1 mg. epinephrin. The synchronous primary fall of pulmonary and carotid pressures, together with a decrease of pulse pressure, indicates that the mechanism is of cardiac origin and not a peripheral change.

Pulsus Alternans.—Pulsus alternans may be a ventricular alternation (more often in hypodynamic hearts) or a concordant atrioventricular alternation. Stimulation of the accelerator nerves is reported to produce pulsus alternans.[24] The changes in rate during alternation are often marked, but their direction is variable. The criteria for the primary fall and secondary rise in such cases depend ultimately upon the steepness and completeness of the systolic volume curves. In concordant atrioventricular alt
weaker beat may pass to a pulsus deficit and occasionally drop f
In one instance there was a sudden permanent omission of the
still effective, beat, causing an instant halving of A-V rate.

Incomplete Systole and Diastole.—This condition is often pr
short period of alternating or irregular beats, after which eve
comes weak with usually, but not always, an acceleration of rate

tricles work from a position of semisystole, i.e., they neither relax nor contract completely. This is evident from volume curves and direct inspection. (See Fig. 6.) Lever methods are entirely inadequate and their records unintelligible in this condition. The heart passes back through the stage of weak or alternating beats to equal strokes on the secondary rise.

The failure of the ventricles to relax and fill properly indicates excessive tonus, for the auricles are full and their systole is apparent upon the ventricular volume curve (Figs. 5, 6). That the condition is not primarily due to

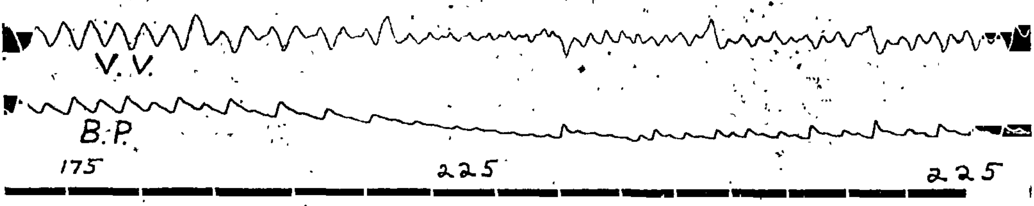


Fig. 5.—Ventricular volume and carotid blood pressure by membrane manometer, time in seconds. Dog, 9 kilo: vagi cut: epinephrin 0.15 mg., fourth dose. The beginning of the primary fall is due to an alternate pulse. The pressure falls farther as every stroke becomes weaker, and the ventricles fail to fill or contract effectively. Other irregularities, not apparent from this single tracing, occur as the secondary rise begins. The pulse becomes alternate again and later equal as the secondary rise progresses. The periodic changes on the volume curve—about every 3 seconds—are due to artificial respiration. The acceleration of rate appears secondary. (See Fig. 6.)

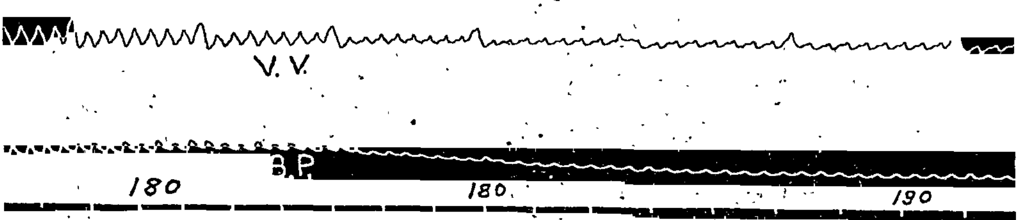


Fig. 6.—Repetition of same dose one hour later than Fig. 5. The inability of the heart to accelerate distinctly during the fall, indicates that in Fig. 5 the acceleration is, at least, not an essential factor. The ventricles are in a position of semisystole or tetany: they neither relax nor contract effectively. The periodic variations on the volume curve, about every 3 seconds, are due to artificial respiration.

increase of rate is evidenced by the fact that the ventricular movements become effective to produce the secondary rise, and often with a further increase of rate.

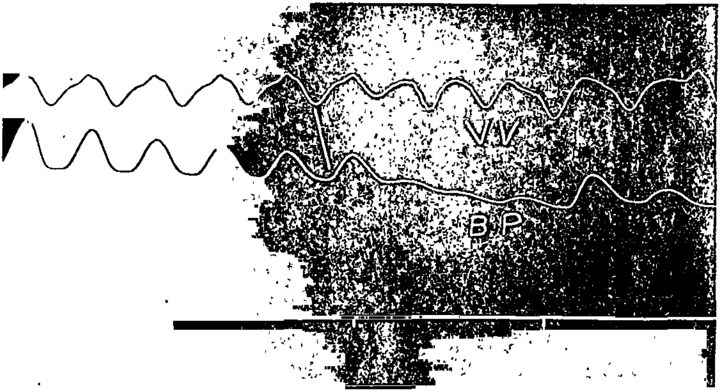
DILATATION

Acute dilatation, instead of incomplete relaxation, often accompanies the ineffective systolic effort (see Fig. 8). This condition is due to dilatation of the left ventricle, because carotid pressure falls before the pulmonary pressure. The pulmonary pressure falls after dilatation is well marked, prob-

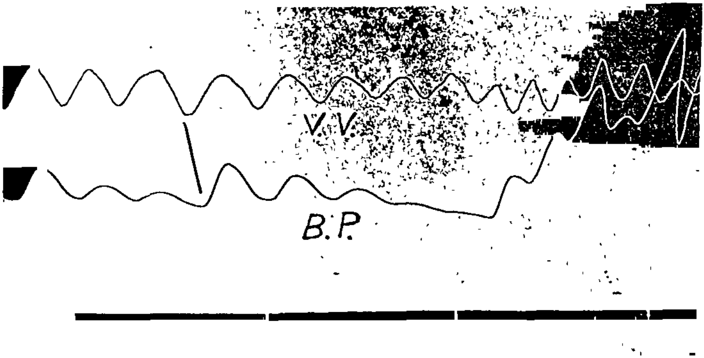
ably from a reduction of filling capacity of the right ventricle, due to the protrusion of the interventricular septum. Acute dilatation occurs more frequently in hypodynamic hearts.

DISCUSSION

As stated previously, the primary fall and secondary rise do not represent a definite mechanism. Likewise, there is no one typical blood pressure curve. The ultimate result represents temporary changes of output, which



A.



B.

Fig. 7.—Ventricular volume and blood pressure by membrane manometer. Dog, 9 kilo: vagi cut: epinephrin, 0.15 mg., previously.

A.—The peak of primary rise and beginning of a primary fall. Note gradual decrease of pulse pressure, followed by irregularities of rate, rhythm, and force.

B.—The beginning of a secondary rise. Note rapid filling and increased velocity of ejection, with a marked increase of rate.

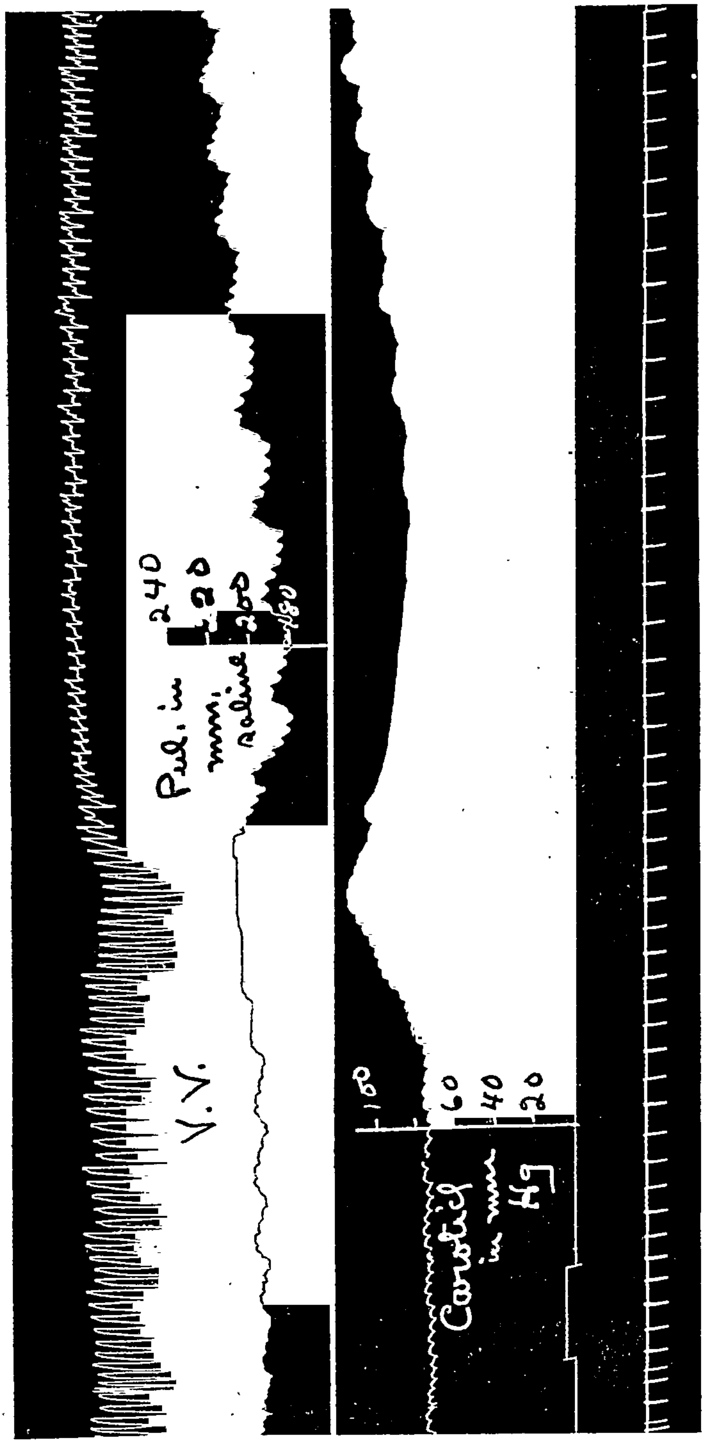


Fig. 8.—Ventricular volume, pulmonary and carotid pressures, time in seconds. Dog, 7 kilo; vagi severed; injection of 0.1 mg. epinephrin. Primary fall due to inadequate systolic effort accompanied by acute dilatation.

are accomplished by alterations in rate, rhythm, and force of the heart beat. The fact that the primary fall is variable and not constant, and also that several factors may operate simultaneously, make the interpretation of the records extremely difficult. Undoubtedly some alterations have passed by unnoticed.

The conditions described as occurring after section of the vagi are attributed to three factors, namely, (1) accelerator stimulation, (2) increased irritability of the heart muscle, and (3) aortic pressure. In ventricular alternation and acute dilatation, the aortic pressure is more important; however, alternation would probably be less prominent if the rate was unchanged during the primary rise. The time relation of the primary fall includes the period of most intense action on the heart from a single injection of epinephrin.

The absence of the primary fall upon elimination of the central nervous system may be explained in part from the observations of Levy,[22] who found that the tendency for cardiac irregularities was diminished under such circumstances.

That obstruction in the pulmonary circuit might cause the primary fall was eliminated in many experiments by simultaneously recording pulmonary arterial pressure (Fig. 4). Schafer and Lim[25] found that the rise of pulmonary pressure on dogs and cats following epinephrin was chiefly of cardiac origin. These authors also observed fluctuations of both pulmonary and carotid pressures, which they attributed to cardiac irregularities. Wiggers[26, 27] found that during the rise of pulmonary pressure after epinephrin, the actual flow in the lesser circuit, as determined by the flow from a small pulmonary vein, was increased.

That epinephrin, or the high blood pressure produced by its injection, may cause a sequential inhibition of rate (vagi severed) seems well established.[2, 15, 16, 17] However, I do not believe the changes are ordinarily so abrupt that the augmented discharge cannot maintain the systolic pressure.

Epinephrin does not seem to have a specific depressing action upon the conduction system. In a personal communication, Carl J. Wiggers expressed an opinion from his own observations that the block produced by epinephrin after section of the vagi was due to so great an acceleration of the auricles that not every impulse is conducted. My experiments tend to confirm this opinion. The A - V intervals in hearts in which incomplete block was produced was abridged from 10 to 30 per cent over normal, following epinephrin until the onset of the block. The A - V interval of hearts which did not develop a block was also shortened following epinephrin. Clinically also, epinephrin appears to improve conduction.[28]

CONCLUSIONS

1. The intravenous injection of 0.1 to 0.3 mg. of epinephrin in dogs produces a blood pressure curve, characterized usually by a primary rise, a primary fall, a secondary rise, and a secondary fall.

2. The primary fall is not due entirely to one definite mechanism, but is usually due to a temporary decrease of minute output in which one or more of the following mechanisms may be operative:

(a) central vagus stimulation,

(b) atrioventricular block,

(c) premature contractions,

(d) pulsus alternans and allied mechanisms representing defective contractile function, and often associated with either

(e) defective filling from abnormal tonus, probably due in part to accelerator stimulation, or

(f) acute dilatation from high aortic pressure.

3. The latter conditions, (b), (c), (d), (e), and (f), may occur also after section of the vagi, but are factors of less importance than the central action.

I wish to acknowledge my indebtedness to Hugh McGuigan for suggestions and criticism of this work and to Dr. Wiggers for information concerning epinephrin block.

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THE RELATION WHICH VARIATION IN SUSCEPTIBILITY AMONG GUINEA PIGS BEARS TO THE ACCURACY OF VIRULENCE TESTS*

By Ruth Gilbert, A.M., M.D., and H. H. Owen, A.B., Albany, N. Y.

It has been suggested by various workers that guinea pigs may occasionally possess some natural immunity to diphtheria toxin.

Theobald Smith,[1] in 1904, stated that variations in susceptibility were the rule rather than the exception and that this condition constituted the most uncertain element upon which toxin-antitoxin tests on guinea pigs depend. He observed that dark guinea pigs were relatively less susceptible than light-colored ones.

Nuttall and Graham Smith[2] mentioned that though variation in susceptibility is not of much importance, it should, nevertheless, be borne in mind as a possible source of error in determining the degree of virulence of diphtheria strains.

Smeeton,[3] in 1917, found that variation in susceptibility of the guinea pigs used exerted a slight influence on the results of her tests. In two instances, animals inoculated subcutaneously showed no reaction, while those inoculated intracutaneously showed marked necrosis. Repeated subcutaneous tests proved the strains to be virulent and the failure of the first tests was probably due to natural immunity of the animals.

Jordan, Smith and Kingsbury,[4] in 1922, reported variations in the reactions to subcutaneous inoculations with virulent strains. They stated that since all the broth cultures used showed good growth, it was evident that some of the guinea pigs employed possessed natural immunity. They also observed that the surviving animals were usually dark-colored.

The study in this laboratory was undertaken to determine whether or not there is sufficient variation in the susceptibility of guinea pigs to prove a source of error in the performance of virulence tests.

According to the routine procedure each strain isolated, which has the typical morphological and cultural characteristics of diphtheria bacilli, is injected intradermally into two guinea pigs, one normal and one immunized with 500 units of diphtheria antitoxin. Four strains are tested on one pair of animals. Each strain which fails to induce a characteristic lesion on the normal guinea pig is injected subcutaneously into another normal animal. In case the guinea pig survives this inoculation and is perfectly well at the end of five days, it is discharged and the strain tested is reported nonvirulent.

For seven months (September, 1923-March, 1924) the susceptibility of each guinea pig which failed to give a definite reaction was tested. During

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this time there were approximately ninety guinea pigs used for the intradermal test. It was necessary to determine the susceptibility of only two of these animals. (On each of the others at least one of the cultures tested gave a definite reaction.) On one of these guinea pigs all four strains tested gave no reaction. Three of these cultures proved virulent when repeated tests were made, both by the subcutaneous and intracutaneous method. The guinea pig which had shown the four negative reactions was tested later with a culture of diphtheria bacilli known to be virulent and only a very weak reaction was obtained. On the other guinea pig, the susceptibility of which was questioned, two strains induced no reaction and two others a weak reaction. Both the latter proved virulent when further tests were made. When this animal was tested with a culture known to be virulent, a weak reaction was again obtained.

Although the possibility of faulty technic cannot be excluded in interpreting these results, it seems probable that these two guinea pigs possessed some degree of immunity.

During the seven months, eighty-five cultures which gave no reaction by the intradermal method were also nonvirulent when tested by the subcutaneous method. The susceptibility of the eighty-five animals used for these subcutaneous inoculations was tested by injecting them with a strain of *B. diphtheriae* known to be virulent. Within two or three days all of these animals died showing the typical lesions produced by diphtheria toxin.

CONCLUSION

Since only two guinea pigs among approximately 175 tested, seemed to show a slight degree of immunity to diphtheria toxin, from a practical viewpoint the variation in susceptibility of these animals does not appear to constitute a source of error in testing the virulence of *B. diphtheriae* according to the procedure described.

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THE MUTUAL RELATIONS EXISTING BETWEEN THE CLINIC AND THE LABORATORY*

By Bowman Corning Crowell, M.D., Philadelphia, Pa.

THE subject on which I have been asked to speak to you this evening is the mutual relations that should exist between the pathologist and the practitioner of medicine. If my remarks are made from the standpoint of the laboratory man, it is because I am best qualified to speak from this viewpoint. I trust, however, that I may be able to emphasize certain phases of the subject that will appeal to the mind of the practitioner, and perhaps aid the worker in deriving the maximum amount of benefit from the laboratory. My remarks will have especial reference to the hospital laboratory, and I may add that, in my opinion, it is essential that relations similar to those that I shall indicate should exist between the practitioner and the so-called private or commercial laboratory if the latter is to perform its function properly.

In the first place, the man in charge of the work in the hospital laboratory today is a development of recent times. He is required to solve problems of many kinds, morphologic, chemical, and humoral, and he must have at his command an insight into many branches of the medical sciences. His primary requisite, however, is a thorough knowledge of the technical methods that may be applied for the detection of diseased conditions. In so far as he is purely a laboratory worker he deals not with the patient, but with the individual elements that go to make up the patient. To him come the blood, urine, feces, gastric contents, spinal fluid, exudates, transudates, or tissues from the patient, but not the patient himself. In order best to perform his proper function he should be able to make the requisite examinations and to indicate the influence, on the patient, of the abnormal conditions that he encounters in his study of the material submitted for examination. There is no need for him to see the patient or to have a knowledge of practical medicine in order to determine that a Wassermann reaction is positive, that the red cells are diminished in number, or that the amount of creatinin in the blood is increased, but he should be able to point out the causes that may be responsible for these conditions, and the bearing that their presence may have on the patient.

But the final word in the diagnosis, treatment, and prognosis of the diseased condition, or conditions, from which the patient suffers rests with the practitioner. It is his function to apply the information furnished by the laboratory to his own interpretation of the condition of the patient, and for this he should not depend upon the laboratory worker. The latter may give him a detailed report of the laboratory findings of the blood, the urine, the

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spinal fluid, or the tissues of the patient, and their variations from the normal, but the formulation of the diagnosis must rest with the clinician. This presupposes a knowledge on the part of the practitioner of the method of interpreting the results of technical procedures.

The purpose of the clinical laboratory is avowedly and properly to aid the physician in his study of the patient, and both physician and laboratory worker should utilize all available means for the advantage of the patient.

Let me indicate the relations that, in my opinion, should exist between the two workers, as well as deprecate some of the present conditions that exist. Their slogan should be *cooperation.* Inasmuch as the aim of both is, or should be, similar, namely, the advantage of the patient, one should help the other to the extent of his ability, and each should act with due consideration for the work of the other, just as two oarsmen should pull their strokes in unison. The problem, therefore, resolves itself into the best method for securing this cooperation. Let me consider, under separate headings, the various factors that go to make up cooperation, it being borne in mind that I speak impartially and not in a spirit of criticism.

1. *When sending material to the laboratory for examination it is well to specify exactly what mode of examination is desired.* The physician's attention is frequently so fully absorbed during the examination of the patient that this simple rule is neglected, and thus much time and labor is lost to the laboratory man. For example, if pus is to be examined for tubercle bacilli, the nature and importance of the examination should be indicated. At times a microscopic examination of a smear for tubercle bacilli is all that is desired, whereas at other times the examination may be of sufficient importance to justify animal inoculation. If, however, this is not indicated at the time the request for examination is made, valuable material that cannot be duplicated may be lost and the laboratory be censured unjustly. Simply to make a request for a bacteriologic examination of a product generally involves unnecessarily a large amount of work. Similarly, the request for "blood examination," so frequently made, is reprehensibly inexact. Under this same heading a word may also be said in regard to leaving laboratory matters to the discretion of internes. I have frequently known a physician, having a leucocyte count in mind, to direct the interne to have a blood examination made, without specifying the type of examination required. Not being quite certain as to what is in the mind of his chief, and desiring to have the information available when called for, the interne directed that all the blood examinations of which he could remember the names at the time be made. True, his chief may thus have been satisfied, but at what unnecessary cost to the laboratory man!

2. *Avoid unnecessary requests.* Examination of the patient should usually take precedence over laboratory investigations. A careful examination of the patient will generally yield information as to what laboratory procedures will best aid in clearing up the case, and in most of the cases only these procedures should be asked for. "Has all laboratory work on the case been done?" This is a question that I have heard repeatedly as the chief was beginning his physical examination. I refer in this place to unnecessary re-

quests because of their inevitable influence on the morale of the laboratory staff. If a laboratory worker has a consciousness that there is a good reason for making every test, his results will, ipso facto, be more reliable and more nearly exact.

3. *The laboratory sciences have their limitations.* This fact should be recognized, and the laboratory should not be held responsible for conditions over which it has no control, or be censured for its inability to do that which is beyond the present scope of the science. This applies perhaps especially to the microscopic examination of tissues. Tissues for examination should be selected carefully, and sufficient information concerning them should be supplied in order to obtain the best results. Frequently if the physician or surgeon is unable personally to visit the laboratory and discuss the case, a brief note on the reverse side of the slip that accompanies the specimen will add a great deal to the information at hand; thus, "This is an interesting case in which there is a question as to diagnosis between a malignant tumor, syphilis, and tuberculosis. The piece is small, it is true, but it is all we could, or will be able to, get." Such a note may lead to animal inoculation for the detection of tubercle bacilli, dark-field examination for treponemata, special fixation for later silver impregnation, in addition to routine morphologic examination. All these would have been impossible if the laboratory man had not thus been forewarned. The absence of such a note has often precluded the possibility of formulating an accurate diagnosis.

4. *The interpretation of results obtained in the laboratory belongs to the province of the physician.* This is true as regards the average case, but, from the nature of his work, in individual or unusual cases the laboratory man is often more skilful in interpreting his results than is the physician, and I have never known a case in which the laboratory worker was not willing to place his knowledge at the disposal of the physician.

5. *Any information that the physician possesses that will aid the laboratory man in making his investigations of a case should be placed at the latter's disposal.* I have known many cases in which such information has intentionally been withheld, at times with the intent of not influencing the laboratory man in making his decision, and at other times because it has been said that, in order to incur favor, the laboratory man would make his diagnosis concur with that of the physician. Of course, such a practice can be regarded only as dishonest. If such a laboratory man exists, he should quickly and forcibly be relieved of his duty. So long as a man is being depended upon to do laboratory work he should be entrusted with all the information that may aid him in his work.

6. *Personal cooperation and consultation between physician and laboratory worker are valuable desiderata.* Personal visits to the laboratory and invitations to the laboratory man to visit the ward and discuss interesting cases cannot fail to be productive of good results to the physician, the laboratory man, and the patient. Real cooperation and an understanding of each other's problems, but not necessarily of one's methods of solution of them, are highly essential. The most successful diagnosticians with whom I

have been acquainted have been men who have made it a practice to visit the laboratory in person with more or less regularity. Whether or not this practice stands in the relation of cause or effect to their success may be a debatable point, but I believe that there is a double relation into which both factors enter.

FROZEN SECTIONS OF TISSUES

Although this topic interests especially the surgeon in his relation to the laboratory, I feel that my presentation of the subject assigned me would be incomplete if this were not referred to. Every laboratory should be equipped for making examinations of frozen specimens of tissue, as the information thus gained cannot be secured in other ways. It is, however, in regard to the advisability and necessity of making frozen sections for diagnostic purposes while an operation is in progress that there is room for a difference of opinion. Personally, it is my belief that facilities for this work should be readily available to every operating room. The cases in which such work should be done as a rapid method of diagnosis for the surgeon is another question. In no phase of laboratory work is cooperation between surgeon and pathologist more essential. In the first place, thorough training in the selection of suitable tissue for examination, and in the interpretation of results secured by this method is a prerequisite on the part of the pathologist. The method is employed most frequently to differentiate tumors and other disease processes, and between benign and malignant tumors, and it is here that this cooperation is most often essential. In differentiating between tumors and other disease processes it is the early cases that give rise to the greatest difficulty, and it is precisely in these cases that numerous foci must be examined carefully before the presence of tumor can be excluded. It is obvious that so careful a search is impossible in the time allowed by a surgeon during operation. In the differentiation between benign and malignant tumors not infrequently the surgeon is in doubt and the pathologist has likewise some difficulty in arriving at a conclusion, since he is restricted in his examination to the small portion of tissue submitted to him. It is on that alone that he can base his opinion. If he finds evidences of malignancy, his verdict is of value. If, however, he finds no evidences of malignancy in the sections he examines, this does not preclude the possibility of malignancy being present. In addition to these groups of cases there are many others in which the pathologist can be of real assistance to the surgeon, and he should ever be ready to lend his aid. My confidence in the discernment of surgeons is great, and I feel that frequently their knowledge of a case is more valuable than any information that the pathologist can furnish them, and their instincts should not be unduly influenced by a doubt raised by a pathologist from the examination of a single specimen, or, at most a few sections hurriedly prepared.

AUTOPSIES

The study of pathologic anatomy has been one of the principal factors in furthering the medical progress that has been achieved since the time of Morgagni, that is, during the latter half of the eighteenth century. It was

Morgagni who first emphasized the importance of comparing the clinical history with the results of autopsy. That autopsies then became frequent is shown by the fact that the classifications of diseased conditions set forth in Rokitansky's "Handbuch der pathologischen Anatomie" in 1846, when he was forty-two years of age, are said to be based on the results of thirty thousand autopsies performed by himself. These figures would tend to show that more than eight autopsies were done every day, including Sundays, during a period of ten years, truly a colossal task. Whether or not these figures are exact, it cannot be denied that this investigator showed an inquisitive spirit of an enviable kind, and set an example that might well be emulated today.

At the present time, in America, the autopsy is not as frequently performed as it should be. For this condition of affairs we as doctors are alone responsible. In Rokitansky's day it was not the layman who demanded the autopsy, but the surgeon himself, who, through his own efforts, obtained the material for autopsies.

Legislation in favor of autopsies may or may not be desirable today, but without specific legislation every physician who attends a dying patient can accomplish more toward securing permission for autopsy than can be achieved in any other way.

The autopsy serves many purposes. Leaving on one side those autopsies that are performed from the medicolegal and sanitary standpoints, let us consider only those that occur in daily practice. More sick and dying are treated in hospitals today than ever before, but *the performance of autopsies should* not be limited to those dying within the walls of a hospital. Nevertheless, it is to this class of patients that the greatest attention in this respect will be paid for some time to come.

It is my purpose to emphasize the advantages that accrue from an autopsy service, and to state some of the methods by which this service may be increased.

Errors in clinical diagnosis occur daily. Cabot's statistics are probably familiar to you all, and if not, they are easily available. Seeking the causes of these errors, we find that they may arise from several sources, which may be classified as follows:

1. Limitations of medical science.
2. Limitations of our individual knowledge of that science.
3. Inaccuracy of observation.
4. Omission of proper diagnostic procedures.
5. Lapses in mental alertness.

It is obvious that each of these causes of error can be diminished by postmortem investigations, but I will endeavor to emphasize this point by illustrative examples. During a cholera epidemic a man entered the hospital with a weak, fluttering pulse, slight rise in temperature, marked prostration, intense abdominal pain, painful diarrhea, and blood-stained dejecta. The patient was at once transferred to the cholera hospital, where he promptly died. The autopsy disclosed the presence of an acute verrucous endocarditis; a calculus impacted in the cystic duct, and bleeding hemorrhoids, but no

cholera. Regarded from some standpoints, this may have been a justifiable error, but, on the whole, it exemplifies a number of the causes of error. Certainly mental alertness, proper diagnostic procedures, and accuracy of observation, coupled with a knowledge of the diseases that were present, might at least have thrown doubt on the diagnosis of cholera.

It is a truism to be remembered that elimination of causes of error leads to greater accuracy in diagnosis, but let us consider this side of the question for a moment and see to what results it may lead. How would the destructive lesion of the chromaffin system in Addison's disease have been found without the aid of autopsies? How would the condition now termed by some a "subacute bacterial endocarditis" have become a distinct entity without the close correlation of accuracy of clinical observation, mental alertness, and postmortem investigations? And how would we know that our clinical diagnoses were frequently inaccurate if we did not have a Cabot to compare clinical and anatomic diagnoses?

I trust that I have emphasized sufficiently the advantages to be gained from postmortem examinations. That these examinations may be obtained with increasing frequency has been strongly exemplified in our own Philadelphia General Hospital. The methods by which this desideratum has been accomplished have been published and can be studied by all who are interested in the subject. I merely wish here to emphasize that an autopsy is an opportunity, and that that opportunity is yours if you care to grasp it. The pathologist can sometimes be of service in the procuring of autopsies, but the real opportunity lies within reach of him who treats the dying and who comes into intimate contact with the family. A word from the chief can often accomplish much more than the arguments or threats of internes, and the interest that each interne has in securing autopsies, is usually in direct proportion to the interest of his chief.

In conclusion, is it not true that among the elements that contribute to progress in medicine anatomic studies occupy an important place? We are now in the midst of an era of progress. Preventive medicine has made habitable some of the waste places of the earth by attention to epidemiologic factors, such as the elimination of breeding places for vectors of disease, and by the more personal, but also more temporary, expedient of mass inoculations with sera and vaccines. The list of acute infectious diseases that can be controlled by appropriate preventive and curative measures is increasing. X-rays and radium are adding to the effectiveness of treatment and strengthening the arm of the surgeon. Chemotherapy is making possible the successful treatment of diseases which were formerly treated symptomatologically. Organotherapy has attained a place for itself in the list of valuable methods of treatment. Now the question may be raised as to whether or not accuracy of diagnosis is keeping abreast of the general progress. It is true that the laboratory with its newly devised biochemic and serologic reactions is aiding in the recognition of some of the more chronic ailments and the x-rays are helping to diagnose hidden foci of disease. But I have already intimated that the laboratory is a mere accessory in diagnosis, which can best serve those who know best what to search for. This brings us back to the man

who can best interpret symptoms and physical signs, for it is to him primarily that we must look for progress in clinical medicine. It may be profitable for us to inquire of ourselves how much progress we are making along these lines today, and as to whether this progress is commensurate with the progress in other branches. If not, is not the man who habitually correlates his clinical findings and the actual anatomic conditions in a better position to contribute to progress than he who does not? Such correlation is possible through the agency of an efficient autopsy service, and this is an opportunity that each of us can make for ourselves.

THE SEDIMENTATION TEST AS AN AID TO DIAGNOSIS AND PROGNOSIS*

By Herman L. Frosch, B.S., M.D., New York

INTRODUCING a new test to our already overloaded diagnostic equipment, should always be done with some hesitancy, and unless it proves to be of decided value, it will naturally be condemned into the archives of medical history. The sedimentation test, I feel and am convinced, will prove of great assistance to the physician, if interpreted in correlation with his other findings.

It was known as far back as our blood-letting ancestors, that the red cells would agglutinate more rapidly in certain diseases than in others, but it was John Hunter who first wrote about the phenomenon, and since then many researchers have studied it carefully, especially in the past three years by the German and Italian workers.

There are several methods of doing this test. The simplest one, however, is the Linzenmeier technic, which is as follows: Into a 1 c.c. syringe which must be absolutely dry, is drawn 0.2 c.c. sterile sodium citrate, 5 per cent strength. To this is now added 0.8 c.c. of blood drawn *directly from a vein.* The contents of the syringe are thoroughly mixed and emptied into specially made tubes, 6.5 cm. long, 5 mm. in diameter and a capacity of a little over 1 c.c. Two marks are used, the 1 c.c. mark and another one 18 mm. below it which is called No. III. The time it takes for the red cells to reach mark No. III and leave the plasma above it clear, is called the sedimentation time.

The test was performed on one hundred consecutive cases at Lebanon Hospital. In order to facilitate the studying of the results, the cases will be divided into four groups.

1. Group I will comprise the cases which had a sedimentation time of one-half hour or less.

2. Group II will comprise the cases which had a sedimentation time of between one-half and one hour.

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3. Group III will comprise the cases which had a sedimentation time between one and two hours.

4. Group IV will comprise the cases which had a sedimentation time of more than two hours.

What application can we make from a study of these groups in surgery, in gynecology, in general medicine?

In surgery, any case having a sedimentation time of one-half hour or less should be operated on immediately, because that is an indication that the inflammation is very marked and that waiting spells danger. Just to cite a few cases to prove this contention.

CASE 1.—D. S., twenty-seven years of age, came in with a history of pain in the abdomen, fever, and vomiting of two days' duration. Physical examination revealed marked tenderness and rigidity in the right lower quadrant; temperature 102°; W. B. C., 14,000; P. 85 per cent, L. 15 per cent. The sedimentation time was twenty minutes, at No. III. On operation an acute gangrenous appendix was removed. Eleven days postoperative, the patient had a normal temperature while the cells sedimented down in two hours to No. III.

CASE 12.—M. K., sixty years old. Three months ago, the man fell and struck his right elbow. Subsequently he developed an abscess in that region, which was incised and drained. The pus gave a Streptococcus hemolyticus growth. The infection did not clear up readily and an x-ray showed an osteomyelitis of the olecranon process with a possible suppurative arthritis of the elbow joint. Temperature was 103° F. while the blood sedimented down in ten minutes to No. III. The man was operated on, but died two days later in pulmonary edema following a solid pneumonic process of his entire left lung.

CASE 68.—M. S., thirty-eight years old, came in with a history of having had pain in the abdomen and fever two weeks previously. The pain was most marked in the right lower quadrant. Three days before admission he had a similar attack, vomited several times, following which he had diarrhea. Physical examination showed marked tenderness and rigidity in the right lower quadrant with an indistinct mass in that region. Temperature 102°; W. B. C. 20,000; P. 78 per cent, L. 22 per cent. Sedimentation time ten minutes at No. III. The operation demonstrated an acute appendix with a lateral cecal abscess and it was drained. Seventeen days postoperative it was still draining pus; temperature 101°; sedimentation time forty minutes at No. III. Thirty-six days postoperative, he had a small sinus which was not draining much, temperature 100°, sedimentation time ninety-five minutes at No. III.

In gynecology if we believe that a case of salpingitis should not be opened up during the acute stage, then a sedimentation time of half an hour or less should absolutely not be operated on.

CASE 45.—J. C., twenty years old, came in with a history of abdominal pain vomiting and fever, menses had been irregular and painful. The day before admission she had a sudden chill. Physical examination showed tenderness and rigidity in the right lower quadrant and slight tenderness in left lower quadrant. Vaginal examination showed the cervix pointing anteriorly, uterus enlarged, and retroverted. Motion of uterus was very painful. There was fulness and marked tenderness in right fornix, and slight tenderness in left. The temperature was 101.4° F., hemoglobin 80 per cent, W. B. C. 13,700; P. 82 per cent, L. 18 per cent, sedimentation time fifteen minutes at No. III. Operation revealed a right and left salpingitis, free pus in the right tube. The appendiceal tip was adherent to the right sided inflammatory mass. Microscopic examination demonstrated a normal appendix while the tubes were acutely inflamed. Seventeen days postoperative, the temperature was normal and sedimentation time was two hours and fifteen minutes at No. III.

CASE 64.—P. L., twenty-two years old came in with tenderness and rigidity in left lower quadrant, fulness in left fornix on examination. Temperature 99°; hemoglobin 70

per cent; W. B. C. 12,000; P. 80 per cent, L. 20 per cent, sedimentation time forty-five minutes at No. III. Operation showed a left salpingitis well walled off from surrounding viscera. The pathologic diagnosis was acute inflammation. Sixteen days postoperative, the temperature was normal and sedimentation time was two hours and thirty minutes at No. III.

No case of adnexal disease should be operated on, unless sedimentation time indicates that all latent foci of infection have been eliminated. Especially should the sedimentation test be used in those cases of adnexal disease which have a normal temperature and normal blood count. A rapid sedimentation time means infection and the operation should be delayed until the test indicates a sterile field.

Can the test be used in general medicine? A study of Tables I, II, III and IV will demonstrate conclusively its importance in medical cases; first, as an aid in prognosis, second, as to the progress of the case. The prognosis should be very guarded in a case having a sedimentation time of less than half an hour. The test done at different intervals of the illness will show whether the patient is improving, or is getting worse. Just to cite a few cases to demonstrate this contention.

Case 2.—M. S., came in with the following history: One week previous to admission he developed precordial pain, sweats and chills and temperature 102° to 103°. He had had frequent sore throats previous to that for which tonsillectomy was performed two months before. Physical examination showed the following: conjunctivitis, right pupil greater than left, systolic and diastolic murmurs at base of heart. He developed transient pains in left knee and right wrist, but no redness or swelling was present. A culture of the blood gave a Streptococcus viridans growth with 500 colonies per 1 c.c. of blood. His temperature was 103° and sedimentation time one hour at No. III. Subsequently he developed a phlebitis of the right antecubital region, bright red blood in his stools, petechial hemorrhages over his lower dorsal region, blood in the urine, splenic infarct and a perisplenitis. His sedimentation time was forty-five minutes. One month later he had a cerebral embolus with a paralysis of the left side of the body and face. Sedimentation time following that was twenty-five minutes at No. III. Four days later the sedimentation time was ten minutes at No. III. He expired four hours later.

Case 3.—M. M., came in with a history of fever, dyspnea and cough four weeks before admission following exposure to cold and rain. He was subject to sore throats three or four times a year. Seven years ago he had an attack of rheumatic fever. Physical examination showed enlarged, red tonsils, systolic and diastolic murmurs heard all over precordium, accentuated P2, pulse deficit 16, and sibilant râles in his lungs. He was fibrillating, temperature 102°; W. B. C. 14,000; P. 83 per cent, L. 17 per cent, sedimentation time was one-half hour at No. III. Three weeks later the patient was clinically very much better. His temperature 99.6° F., sedimentation time one hour at No. III. Blood culture was sterile. Four weeks later the temperature was normal and sedimentation time one and one-half hours at No. III.

Briefly, an increasing sedimentation time in a patient means improvement. A diminishing sedimentation time means that your patient is getting worse, even though the clinical picture indicates improvement.

The temperature does not in any way affect the sedimentation time. In one of the typhoid cases with a temperature of 104° to 105° F. the sedimentation time was one and one-half hours at No. III, while in a patient with an acute appendicitis whose temperature was 100° F. the sedimentation time was twenty minutes at No. III.

GROUP I

CASE	DIAGNOSIS AND CLINICAL CONDITION	SEDIMENTATION AT DIFFERENT INTERVALS IN MINUTES TO NO. 3 AND COMMENT	
1	Acute gangrenous appendicitis	20 min.	11 days P.O.*—120 min.
77	Secondary appendiceal abscess	20 "	Before discharged, 90 min.
11	Acute appendicitis with general peritonitis	15 "	7 days P. O.—105 min.
13	Acute appendicitis	15 "	11 days P. O.— 90 min.
33	Acute appendicitis with general peritonitis	15 "	1 day P. O. 30 min. 18 days P. O. 150 min.
41	Acute gangrenous appendicitis	25 "	1 day P. O. 60 min. 13 days P. O. 135 min.
53	Acute appendicitis		2 days P. O. 30 min. 10 days P. O. 65 min.
68	Acute appendicitis with lateral cecal abscess	10 "	17 days P. O. 40 min. 36 days P. O. 95 min. Drain in small sinus
82	Acute appendicitis	105 "	Patient not operated
86	Acute gangrenous appendicitis with local peritonitis	20 "	1 day P. O. 35 min. 10 days P. O. 70 min.
96	Acute appendicitis	13 "	1 day P. O. 52 min. 13 days P. O. 105 min.
3	Acute rheumatic endocarditis fibrillating, left pulmonary infarct, sore throat. Blood culture sterile	30 "	3 weeks later 60 min. 4 weeks later 90 min. Patient discharged
8	Perirectal abscess	12 "	7 days P. O. 120 min.
10	Chronic myocarditis, auricular fibrillation, embolism left femoral		Amputation of left leg, right sided complete hemiplegic attack, 30 min. 3 weeks later 120 min.
12	Suppurative arthritis and osteomyelitis	10 "	Patient died
18	Thromboangiitis obliterans, infection right leg, amputation of right leg	13 "	9 days P. O. 10 min. 31 days P. O. 125 min. Stump infected
20	Double aortic and mitral regurgitation; acute rheumatic fever;	26 "	1 day before death 10 min.
23	Internal hemorrhagic pachymeningitis	30 "	Before discharge 150 min.
28	Cardiac insufficiency, chronic myocarditis, subacute rheumatic fever, pneumonia	10 "	10 days later 12 min.
29	Carcinoma of stomach	18 "	
31	Subaponeurotic abscess of scalp	15 "	11 days P. O. 45 min. 14 days P. O. 90 min.
36	Acute articular rheumatism	14 "	
38	Persistent obstipation and vomiting; severe cold	15 "	
40	Polyirectal abscess; pulmonary tuberculosis	22 "	
45	Bilateral pyosalpinx	15 "	17 days P. O. 170 min.
46	Lobar pneumonia, type IV	13 "	10 days later 190 min.
47	Acute rheumatic fever	15 "	3 weeks later 105 min.
48	Abscess of lung	15 "	5 weeks later 45 min.
50	Cardiac insufficiency; chronic myocarditis; bronchopneumonia	22 "	Expired
56	Cellulitis of forearm	30 "	4 days later 135 min.
58	Chronic cholecystitis; cirrhosis of liver	15 "	
59	Complete general arteriosclerosis	25 "	Later 45 min.
60	Diabetes; cardiac hypertrophy; chronic bronchitis and myocarditis decompensated	25 "	
78	Cellulitis	15 "	2 days P. O. 25 min. 8 days P. O. 65 min.
81	Common duct obstruction and jaundice	28 "	
89	Nephrolithiasis with pus	25 "	
93	Pyonephrosis	18 "	2 days before death 10 min.
95	Lobar pneumonia	11 "	1 week before discharge 180 min.
64	Left salpingo-oophoritis	22 "	7 days P. O. 45 min. 16 days P. O. 135 min.

*P.O.=postoperative.

Is the sedimentation test any improvement on our usual blood count? I think so. One case will demonstrate how the test proved to be right.

CASE 11.—J. B., came to the hospital with a history of vomiting, abdominal pain and fever of two days' duration. Physical examination showed tenderness and rigidity in the lower half of the abdomen. Operation revealed an acute appendicitis with a general peritonitis. Four days postoperative his temperature came down to normal and stayed there until eleven days after operation when it went up to 102° F. Examination revealed tenderness in right flank. A blood count showed W. B. C. 10,000; P. 67 per cent, L. thirty-three per cent. Certainly his blood count does not indicate an inflammatory condition. But a sedimentation test taken the same day showed that the red cells came down to No. III in fifteen minutes. On opening the abdomen an abscess was found situated between the parietal peritoneum, the cecum and ascending colon containing thick greenish pus. The temperature came down promptly. One week later the sedimentation time was two and three-fourths hours at No. III.

GROUP II

CASE	DIAGNOSIS AND CLINICAL CONDITION	SEDIMENTATION AT DIFFERENT INTERVALS IN MINUTES TO NO. 3 AND COMMENT	
2	Acute endocarditis, aortic stenosis and regurgitation, blood culture Streptococcus viridans	60 min.	3 weeks later splenic infarct and perisplenitis 45 min. 2 months later cerebral embolus 25 min.; died
4	Pyelitis	35 "	4 days later 25 min., 7 days later 90 min.
6	Chronic cholecystitis and chronic appendicitis	40 "	
14	General intraabdominal adhesions and chronic bronchitis	40 "	
15	Suppurative axillary adenitis	55 "	3 days P. O.* 105 min.
27	Typhoid fever	53 "	
42	Medial ventral hernia	50 "	12 days P. O. 175 min.
76	Acute gastroenteritis	55 "	
85	Incomplete abortion	45 "	
63	Chronic appendicitis	60 "	11 days P. O. 115 min.
84	Intramammary abscess	55 "	2 days P. O. 115 min.

*P.O.=postoperative.

GROUP III

CASE	DIAGNOSIS AND CLINICAL CONDITION	SEDIMENTATION AT DIFFERENT INTERVALS IN MINUTES TO NO. 3 AND COMMENT	
9	Right indirect inguinal hernia, and hydrocele of canal of Nuck	85 min.	2 days P. O.* 60 min. 12 days P. O. 150 min.
37	Typhoid fever	90 "	
43	Calculus in ureter	115 "	
44	Neurosis of intestines	120 "	
52	Cellulitis of face	90 "	
54	Fracture of right tibia	80 "	
57	Acute arthritis of left hip	80 "	
61	Ruptured duodenal ulcer		1 day P. O. 90 min.
65	Salivary calculus; and acute suppuration, sublingual adenitis	105 "	
70	Laceration and crushing of fingers	110 "	
75	Syphilis of liver, hemiplegia	105 "	
79	Contusion of right leg	93 "	
80	Undetermined diagnosis	105 "	
83	Fracture of tibia and fibula	85 "	
91	Chronic appendicitis	100 "	
92	Fracture of rib	120 "	
94	Resolving lobar pneumonia	65 "	
73	Acute suppurative adenitis	75 "	4 days P. O. 135 min.
99	Typhus fever(?)	90 "	Four days later 135 min.

*P.O.=postoperative.

GROUP IV

CASE	DIAGNOSIS AND CLINICAL CONDITION	SEDIMENTATION AT DIFFERENT INTERVALS IN MINUTES TO NO. 3 AND COMMENT	
5	Lipoma of neck	240 min.	
16	Hemorrhoids	190 "	
17	Compound fracture of skull	125 "	
19	Mitral stenosis	150 "	
22	Partial heart block	187 "	
24	Acute nephritis and myositis	180 "	
30	Gastrojejunal ulcer	125 "	
32	Left oblique inguinal hernia	125 "	
34	Fistula in ano	360 "	
35	Contusion of left side of face	220 "	
39	Hemorrhoids	390 "	
49	Hemorrhoids	240 "	
51	Influenza		Five days before discharge 300 min.
55	Neurosis of heart	300 "	
62	Neurosis of stomach	405 "	
66	Probably chronic appendicitis	150 "	
67	Fracture of skull	135 "	
69	Chronic appendicitis(?)	480 "	
71	Hemorrhoids	180 "	
74	Fracture of tibia and fibula	195 "	
77	Hemorrhoids	540 "	
87	Nephrolithiasis	135 "	
88	Indirect inguinal hernia	150 "	
90	Cystic hypertrophy of endometrium	135 "	
97	Chronic interstitial nephritis	210 "	
98	Chronic myositis, tonsillitis, and pharyngitis	480 "	

In order to determine which portion of the blood causes the change in the sedimentation time, that is, whether the cause is in the plasma or in the red blood cells, the following experiments were performed:

The serum of a patient whose sedimentation time was ten minutes was intimately mixed with the red cells of a patient whose sedimentation time was two and one-half hours in normal proportions. This mixture sedimented down to No. III in twenty minutes. The reverse experiment was also performed by adding the serum of the latter case to the cells of the former. In this case the sedimentation time was two hours and ten minutes at No. III. This type of experiment was repeatedly performed and gave the same results. The only conclusion that can be derived from the above, is that the rate of sedimentation is dependent upon some constituent in the plasma rather than in the red cells.

It is a well-known fact that in certain diseases as pneumonia, acute articular rheumatism, the various suppurative conditions, and in diseases where tissue destruction is occurring there appears in the plasma of the blood a relative increase in globulin and fibrinogen, with a relative diminution in albumin. This change results in a diminution of surface tension, which also means a diminution in the cohesive powers between the individual molecules making up the plasma, and thus the red cells come down faster than in a normal individual.

There are at present three outstanding theories as to the cause of the quickening of sedimentation in certain diseases.

Fahrenaus and Huber regard it as due to a change in the electric charge of the red cells. Normally they carry a negative charge and thus they repel

each other. In certain diseases this negative electric charge is diminished. This diminishes the repelling qualities for each other, facilitating agglutination and sedimentation.

Plaut seeks an explanation of this phenomenon in increased autoagglutination which he thinks is due to an increased fibrinogen content of the plasma.

Sachs claims that it is due to a variation in the stability of the colloids of the plasma in which globulin and fibrinogen are the most changeable components.

Wilhelm Starlinger performed a very clever and ingenious experiment a few years ago. He added kaolin, which absorbs fibrinogen to a blood whose sedimentation time was very rapid. He then showed that the sedimentation time of that blood was very much increased after such procedure.

After a thorough study of the literature as to the behavior of colloids, it seems to me that all three theories are correct. Primarily, there is a relative increase of globulin and fibrinogen in certain diseases. These particular colloids are composed of larger molecules than the other usual colloids of the blood. Thus, there is a diminution in the cohesive power between the molecules making up the plasma. Furthermore, these colloids also have a greater absorption power for the alkaline salts in the plasma and thus the negative electric charge of the red blood cells is diminished, which is conducive to more rapid agglutination of the cellular elements of the blood. Briefly, an increase in globulin and fibrinogen produces a lower surface tension of the plasma and a more rapid agglutination of the red cells, and thus a more rapid sedimentation time in those conditions in which these two colloids are increased in the plasma.

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A COMPARATIVE STUDY OF THE KOLMER COMPLEMENT-FIXATION TEST FOR SYPHILIS WITH SERA BEFORE AND AFTER THE REMOVAL OF ANTISHEEP HEMOLYSIN*

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FOR many years, there has been diverse opinions as to the value of absorbing the natural antisheep hemolysin from sera before conducting the complement-fixation test for syphilis. That an appreciable amount of antisheep hemolysin is present in human sera has been demonstrated by numerous investigators. A detailed résumé has been compiled from the available literature on this subject by Sachs.[1] Kolmer and Casselman[2] have titrated the thermostabile hemolysins for corpuscles of various vertebrates in a large number of specimens of human sera. They observed that in over 80 per cent of these sera, there is present sufficient natural antisheep hemolysin to give well marked or complete hemolysis. Van Saun[3] obtained similar results in a large proportion of cases (51 per cent). Working with a series of over 6,000 samples of sera which had been submitted for routine complement-fixation tests for syphilis, only 12 per cent were found by Gilbert and Wemple[4] to contain appreciable amounts of natural amboceptor (1924). Our own results are more nearly in accord with those of the former, our percentage being approximately 60 per cent.

Bauer[5] and Hecht-Weinberg[6] therefore advised the modification of the Wassermann reaction on those samples of sera already containing the proper dose of hemolytic amboceptor, whereby this natural amboceptor is utilized, with no need for the addition of an immune amboceptor. In this modification, none of the syphilitic antibody is destroyed and no complementoid produced. Kolmer proved that the Hecht-Weinberg modification is quite delicate because of this, but claims that false complement fixations occur when crude alcoholic extracts of the organs are used as antigens. To obviate this Noguchi[7] made use of an antihuman in place of the antisheep hemolytic system; but he overlooked the possibility of a natural antihuman hemolysin, which Williams[8] has shown to be present in some sera.

The question therefore arises: Shall or shall we not absorb this natural antisheep hemolysin before adding the artificial hemolytic system? Is the sensitiveness of the test affected by its presence or absence? This we have endeavored to answer by conducting a series of experiments whereby the complement-fixation test was performed on human sera prior to and after the absorption of the natural antisheep hemolysin. We also wished to confirm or deny the results of other workers who had not used Kolmer's new

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method for complement fixation with and without this absorption. While some investigators have found that the effect of the natural amboceptor is so slight that it can be considered negligible, there are others who quite disagree with this conclusion. Much depends upon the factors of technic employed, such as the test of serum complement and amboceptor, the duration of the primary incubation period and the time at which the final reading is made, whether at once or after standing several hours or overnight.

Kolmer and Rule[9] have studied in detail the factors affecting the ultimate readings of the test. Strongly positive sera, containing large amounts of syphilitic reagin are not influenced in quantities of 0.1 and 0.2 c.c., by the presence of the antisheep hemolysin naturally contained in the sera. However, sera with relatively small amounts of syphilitic antibody and large amounts of natural amboceptor may yield falsely negative reactions, unless the natural hemolysins are removed. The effect upon weakly positive sera is not as pronounced when the readings are made immediately after the second period of incubation, as when they are made after the tubes had been placed in a refrigerator for sixteen hours or longer.

A recent paper has appeared by Gilbert and Wemple[4] in which are reported similar results to those given by Olmstead[10] and Kolmer and Rule.[9] They are agreed that the influence of natural amboceptor is less apparent if the complement-fixation tests are read immediately after the second incubation than if read the next morning. But Olmstead[10] concluded from the researches that if the tubes are read as soon as the serum and antigen controls are completely hemolyzed, the natural amboceptor content of the serum makes but little difference in the Wassermann test. The removal of natural hemolysin, in his opinion, does not appear to be essential to the sensitiveness of the test.

Those[7, 23, 24] who employ an antihuman hemolytic system appear convinced that it is more sensitive owing to the absence of these natural antisheep hemolysins, in contradistinction to those who, using the antisheep hemolytic system, deny or minimize the influence of the natural amboceptor in the technic to which they adhere. The addition of artificial antisheep hemolysin is unnecessary, owing to the natural amboceptor present, for the production of hemolysis in the serum control tubes. Theoretically, at least, the presence of unknown natural amboceptor in some sera, will obscure evidences of its quantitative relations to the antisheep hemolytic system, and the reactions may still be inaccurate. There are those, therefore, who deem it a more satisfactory solution of the problem to remove the natural hemolysin from the sera before the performance of the test, so that the balance of the hemolytic system may be assured by the addition of the known quantity of amboceptor.

It must be remembered that the authors cited above used the Wassermann complement-fixation technic. Only one investigator (Kilduffe[11]) applied the absorption of the natural amboceptor to Kolmer's new method so widely advocated. This technic is characterized by a very high degree of specific sensitiveness, by almost no tendency to yield apparently nonspecific reactions, and by a very low percentage of anticomplementary reactions

(Schamberg and Klauder,[12] Shivers,[13] Kilduffe,[14] Palmer and Gibbs,[15] Smith[16] and others).

It might not be amiss to give in detail Kilduffe's[11] conclusions. I may add that our own experiments were in progress when these results were published. It was, therefore, of great interest to us to learn whether or not our findings tallied with his. He states, "The presence of large amounts of natural antisheep hemolysin is not without effect upon the reaction in Kolmer's quantitative complement-fixation test for syphilis, even when the serum contains relatively large amounts of syphilitic reagin, and may give rise to false negative results in approximately 10 per cent of hemolysin containing sera." He thus concurs with the opinion of those who had used the old method, and advises that the natural antisheep hemolysin be absorbed from the serum prior to the performance of Kolmer's quantitative complement-fixation test, and so increase the delicacy of the reaction.

EXPERIMENTAL

In all probability, the complement-fixation test for syphilis serves its most useful purpose in the diagnosis of the disease in its clinically latent and tertiary stages, as a guide in treatment and a criterion of cure. The very nature of the pathologic and immunologic changes in chronic and latent syphilis demands that the complement-fixation test shall be consistent with specificity to render its best service, as an aid in diagnosis. Therefore, it is essential to determine the exact part the antisheep amboceptor plays in the sensitiveness of the test. Before ascertaining this, we thought it necessary to confirm the findings of other workers as to the percentage of human sera found to contain antisheep hemolysin (0.1 c.c. human serum was used throughout in the serum control). We had at our disposal, numerous patients from the out-door department of the Polyclinic Hospital, of Philadelphia, which included specific and nonspecific cases, the former comprising all stages of the disease from the frank secondaries and primary to the tertiary and latent syphilis. Many methods for the absorption of antisheep hemolysin are in vogue. Among the earlier workers were Bauer,[17] Rossi[18] and Jacobaeus.[19] They advocated the use of homologous corpuscles, but this procedure, according to Bauer,[17] frequently rendered the serum anticomplementary. This is known as the Sachs-Friedberger phenomenon, and is counteracted by the absorption of heated sera at a low temperature (Rossi[18]). Rossi, therefore, makes use of the refrigerator in the active part of the absorption. All investigators in this phase of the work utilize sheep's cells, but each in turn had so modified the original procedures as to render them rather time consuming and laborious. Among the latter is Simon[20] who advocates the use of varying amounts of serum in his test. This method has been used by Kolmer, who found it very satisfactory, showing but an occasional anticomplementary result. This latter unfavorable condition has been combated by Kahn[21] in his excellent method of allowing the absorption process to take place in but ten minutes at room temperature, followed by centrifuging and storing in the refrigerator for sixteen to eighteen hours. This technic has been highly recommended by Kilduffe.[11] Gilbert and Wemple[4] described a

similar nontime-consuming procedure which they claim produces no anticomplementary properties; they make use of the refrigerator as does Rossi (3° 6° C.).

The ideal method for removing antisheep amboceptor from human sera should be efficacious, rapid and simple, and with the added attribute of not rendering the sera anticomplementary. We have attempted to devise a method which would come closest to the required properties. Our technic was as follows: A series of 500 sera was tested for the presence of antisheep hemolysin. The serum of each specimen to be examined was divided into 2 equal parts. One portion was tested "untreated" but inactivated at 55° C. for fifteen minutes. The other was subjected to the following: To 1 c.c. of serum we added 2 drops of washed well-packed sheeps' cells (from a 1 c.c. pipette); the mixture was allowed to remain at room temperature for fifteen minutes. After centrifuging to separate the "treated" serum from the cells, this serum was inactivated at 55° C. for fifteen minutes. It now remained to test whether the natural antisheep amboceptor had been absorbed. To 0.1 c.c. of the "treated" serum was added 1 c.c. of 2 full units of complement and sufficient isotonic salt solution to make 2 c.c. The next day, after the mixture had remained in the refrigerator for sixteen to eighteen hours, 0.5 c.c. of a 2 per cent suspension of sheep cells was added and incubated for one hour at 38° C. (It will be noted that no artificial hemolysin was used.) Reading of the tube was made immediately after incubation. There was complete inhibition of hemolysis showing that the hemolysin for the corpuscles employed was absent. As a control, the untreated inactivated serum was subjected to the same procedure, with resulting complete hemolysis in the majority of the cases, i.e., in those sera which contained a sufficient amount of natural antisheep amboceptor. This absorption method satisfied all requirements and was used throughout the experiment.

Before the above technic was finally decided upon, we experimented with varying periods of sensitization of sheep cells and active serum; we obtained frequent unsatisfactory results in those cases in which sensitization took place for more than twenty minutes, owing to discoloration of some sera. Good results were obtained with contact at fifteen minutes, which procedure we adhered to.

To return to our comparative study of the complement-fixation reaction of the new Kolmer technic for syphilis, with sera before and after the removal of antisheep hemolysin. We now subjected the "treated" and "untreated" sera simultaneously to the standardized Kolmer method, using the cholesterinized lecithinized antigen with 1 c.c. of two full units of complement and with the proposed primary incubation period of sixteen to eighteen hours at 6° to 8° C., followed by five to ten minutes in the water bath at 38° C. The hemolytic system comprising 0.5 c.c. or two units of hemolysin and 0.5 c.c. of 2 per cent suspension of sheep cells was added prior to the second incubation period of one hour at 38° C. The tubes were read immediately thereon.

It was interesting to investigate the clinical data of the cases from which the sera with negative complement-fixation results had become positive after the natural amboceptor had been removed. Table IV gives the relation between the clinical diagnosis and the corresponding sera yielding positive sera after absorption had taken place, but negative sera before. There were 40 cases examined, which embraced nonsyphilitics, suspicious cases as well as patients suffering from all stages of lues. Twenty-one of the total number of 40 were latent syphilitics. In all of these, the positive reaction was elicited after "treatment" of the sera in contrast to the negative results prior to this removal of the amboceptor. Tests were conducted on sera withdrawn on separate occasions from the same patients later found to be suffering from various stages of lues. Protocols are given in Table V and show that these positive reactions after absorption were not sporadic, but remained consistent in the various tests made at periodic intervals. All these luetic patients, most of whom were latent syphilitics, were receiving treatment during the conduction of these experiments.

DISCUSSION

From our experiments, we deducted the fact that of all the fresh inactivated human sera examined, a large proportion contained sufficient natural hemolysin to completely or markedly hemolyze the usual dose of sheep cell suspension with its customary amount of guinea pig complement. There is a difference of opinion, however, as to the exact percentage of cases containing natural hemolysin, some workers claiming that there is only a small and insignificant number of such sera. This diversity of opinion is probably due to the different technics followed, varying amounts of complement and sheep cells used, and whether short or long incubation periods had been advocated. It will be readily understood that the presence of an unknown amount of natural antisheep hemolysin in sera would be a drawback to accurate quantitative estimations. Even an excess of 4 or 5 units of antisheep hemolysin will so influence the reactions as to cause an occasional false reaction to occur or a positive result to be lost (Noguchi,[7] Olmstead,[10] Bailey[25] and Kolmer[9]).

It is the opinion of Kolmer and Rule[9] that an appreciable amount of natural hemolysin in the presence of large quantities of syphilitic reagin will be insufficient to mask the detection of complement-fixing bodies. These findings have been confirmed by Kilduffe.[11]

The results of our own experiments (Table III) show that out of the 40 samples of sera which gave positive results after the removal of the hemolysin and negative before, 23 were weakly positive in the first tube. We therefore concluded that small amounts of syphilitic reagin are masked by the large amount of antisheep hemolysin naturally found in sera. The remaining 17 tubes showed a moderately positive reaction. The latter thus contained relatively larger amounts of syphilitic reagin and were also influenced by the natural amboceptor. The results of the complement-fixation tests (Kolmer's new method) on all the 40 sera specimens, before "treatment," were negative. suggesting the conclusion that whether small or large

amounts of syphilitic reagin are contained in the sera, the presence of natural antisheep hemolysin will affect the readings.

This mechanism is due to one of two explanations. It may lie in the fact that for some unknown reason an excess of hemolysin may completely hemolyze the corpuscles, even though a small amount of the necessary complement has been specially fixed by the antigen and the syphilitic antibodies, or it may be due to the fact that large amounts of hemolysin prevent some of the complement from joining with the antigen and syphilitic antibodies, and this renders the complement free. It is the opinion of a number of research workers that absorption of the sera does not remove the syphilitic reagin.

Two hundred and sixty-three of our series of 500 revealed positive reactions before removal of the antisheep amboceptor. Six of these showed weaker positive results after treating the sera with sheep corpuscles, but falsely negative readings after absorption were never obtained throughout the experiment.

These weaker positives are probably due to other factors rather than heating to 55° C. It is not avoidable to eliminate errors attributable to slight inaccuracies from pipetting the exact quantities indicated. Such diminutive amounts of packed sheep cells are used (2 drops) that any salt solution added in the washing of the corpuscles that had not been removed, may so dilute the serum as to mask the reaction. Care should be taken to rule out this possibility and to discard as much of the saline as is possible. In spite of this caution Van Saun[3] claims that the antibody content of the serum in a few instances is weakened by the absorption treatment.

It is generally admitted that there may be a development of anticomplementary properties in an occasional serum after absorption with sheep corpuscles. Some little difficulty has been encountered in finding a method satisfactory for the complete absorption of hemolysin without the coincident development of the thermostabile anticomplementary properties in the serum after absorption. Sach's explanation of the Sachs-Friedberger phenomenon is that the serum possesses a so-called antagonistic nature in addition to the natural hemolysin. This nature is produced by the "Hemmende anti-Korper" and causes the anticomplementary activity, being enveloped by the normal amboceptor.

According to Rossi, methods advocating the use of incubator temperature removes only the normal amboceptor. Low temperatures for sensitization do away with both the natural hemolysin and this antagonistic nature of the serum. His recommendation of low temperature rests on this factor. Kahn advocates ten minutes at room temperature followed by storing in the ice chest overnight and claims to have eliminated anticomplementary results with this procedure. We have usually removed anticomplementary substances by heating the "treated" sera to 55° C. for fifteen minutes immediately prior to the complement-fixation test. We have been able to demonstrate that only 31 out of the 500 sera specimens of our series or 6 per cent contained thermostabile antilysin which was not influenced by fifteen minutes exposure to 55° C. From the viewpoint of Rossi it may be that low

temperature for the sensitization of the sera may be advisable, in spite of the extra time involved.

Two methods have been advocated for the absorption of the natural antisheep amboceptor by the sensitization with sheep cells, the main differing factor being the use of active or inactivated sera. It is conceded that, by subjecting the sera to the fifteen minute exposure to 55° C. for inactivation, some of the thermolabile reacting bodies contained in the sera are destroyed. The second heating to 55° C. used in the process of absorption further removes those bodies which had escaped the first heating. The total exposure to 55° is not more than thirty minutes, no longer than that formerly in use in the Wassermann technic. We have employed only active sera in our sensitization process with sheep cells and have allowed extraction to take place at room temperature instead of the refrigerator; for it is our belief that the one heating to 55° for fifteen minutes during the period of absorption is sufficient to remove the native natural complement in human sera, with no production of complementoid. The thermolabile reacting bodies are thus little destroyed. The sensitization of the sheep's corpuscles is more rapid when taking place at higher temperatures than at lower. The length of time allowed for this is important, owing to the likelihood of discoloration occurring in the sera when there is too long an elapse of time. After experimenting with different time intervals, we found that the fifteen minutes at room temperature is best for our purpose, thirty minutes causing a tinge of discolor in the sera.

Although we must admit that we have obtained an occasional anticomplementary reaction, there is no doubt that our technic of the removal of natural antisheep hemolysin does increase the sensitiveness, and enhance the delicacy of the complement-fixation test. With further concentration to perfect the method this disadvantage may be counteracted.

It has been observed that the Kolmer new standardized technic will yield an occasional anomalous reaction. In a definite proportion of these anomalously reacting sera, the reaction is due, according to Kolmer, partly to the presence of relatively large amounts of natural antisheep hemolysin (although he has found them to occur in its absence), and partly to the serum constituents, the nature and mode of which are as yet unknown. Kilduffe has offered experimental data confirming this. Our own findings, based on 500 samples of sera reveal but one anomalous reaction before and one after absorption. Such anomalous reactions do not, therefore, seem to depend upon the presence or absence of natural amboceptor. We believe, with Kolmer and Kilduffe, that the serum constituents play an important rôle in these reactions.

To judge the delicacy of any modification of the current complement-fixation test. clinical findings must be correlated with the laboratory reports. We set out to investigate the diagnostic findings of the 40 patients whose sera gave a positive reaction after absorption and a negative one before. The majority of these were latent syphilitics. Sera obtained from these at different intervals were consistent in giving inhibition of hemolysis after treatment of the sera. and noninhibition before. Kolmer and Steinfield[22]

propose as an explanation for false negative reactions on inactivated, untreated sera, that the blood of latent syphilitics contain little or no syphilitic reagin, at best, an insufficient quantity to fix the complement and specific antibody. Removal of the natural amboceptor, rendering the test more delicate, will aid in the laboratory diagnosis of these cases. It is our contention, therefore, that in spite of the additional time necessary for the absorption, the removal of the natural antisheep hemolysin is not only of theoretical, but of practical importance. We cannot hope to detect all cases of syphilis by the complement-fixation or any other test known today, but it is our duty to reduce the incidence of false negatives to a minimum by *employing a test which will yield reactions of as maximum a degree of* sensitiveness as is consistent with specificity.

Sera of probable latent syphilitics which contain large amounts of natural hemolysin and react negatively to the known complement-fixation tests of today, giving a further negative reaction after removal of the natural amboceptor, are believed by us to be not indicative of syphilis and will never be biologically so proved.

SUMMARY

1. Human sera frequently contain relatively large amounts of natural antisheep hemolysin. Antisheep hemolysin in sufficient quantities may serve as a source of error in the complement-fixation tests. An excess of natural antisheep hemolysin in human sera may yield falsely negative results, even though the sera may contain relatively large amounts of syphilitic reagin.

2. An appreciable difference in the readings of the complement-fixation tests are observed before and after the removal of natural antisheep hemolysin. The sensitiveness of the reaction is increased when the sera are "treated" and deprived of the amboceptor.

3. An occasional thermostabile anticomplementary activity is obtained with our method of absorption.

4. The Kolmer standardized technic for the complement-fixation test, in conjunction with hemolysin free sera, serves as a more delicate test than when used on simply inactivated sera.

5. The absorption of the natural antisheep hemolysin is most useful in the complement-fixation test on sera from latent syphilitics. It is in the cases of falsely negative results that special attention should be paid to measures which will not mask the syphilitic reagin.

6. In spite of the occasional anticomplementary reaction obtained after absorption, it is our opinion that "treatment" of all sera should be undertaken before the conduction of the complement-fixation test. A more reliable method for removing antisheep hemolysin from human sera should be looked for than that in current use.

I wish to express my gratitude to Dr. John A. Kolmer for suggesting and advising me upon this problem. To Dr. S. Greenbaum, I am indebted for supplying the clinical data. I am thankful to Doctor Dorothy Wilkes-Weiss for assisting me in the preparation of this paper.

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CHANGES OF IRRITABILITY IN WOMEN DURING THE MENSTRUAL CYCLE*

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General observations seem to indicate that there is a variation in the general bodily tone in women during the menstrual cycle. This question has already been investigated by King,[1] Jacobi[2] and others. The data presented up to the present time seem to indicate that there is a period of hyperirritability immediately preceding the menstrual period which gradually returns to normal during the intermenstrual period.

The question arose in this laboratory, while making a comparative study of the irritability of men and women, whether it was necessary to consider menstruation in case of the women. In order to throw more light on this question it was decided to carry out the investigation herein reported.

Since it has been shown by the writer and numerous other investigators that whatever influences the general tone of a mechanism markedly affects the knee-jerk, it was decided to use this reflex as an index to any nervous change which might take place during menstruation. King[1] carried out an investigation similar to this and obtained results which, in general, supported those already obtained.

Data were collected from five women in good health ranging in age from twenty-five to thirty-five years. In all cases menstruation was regular and

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normal, causing them no unusual discomfort. Each subject came to the laboratory at a definite hour daily during a complete menstrual cycle.

The cycle was divided into periods as follows[3]: (1) period of growth, five days preceding menstruation; (2) period of menstruation, approximately four days, or as reported by the subject; (3) period of regeneration, seven days; (4) period of rest, twelve days.

The experimental conditions were kept as constant as is possible in knee-jerk experiments. The subjects were adjusted in the apparatus[4] especially constructed for knee-jerk investigation and allowed to deliver jerks for twenty minutes. As soon as the adjustments were made, the operator left the laboratory and the subject remained alone throughout the experiment. The stimuli were delivered to the center of the ligamentum patellae at the rate of seven per minute. The exact point of impingement was carefully marked in each case in order to eliminate the possibility of varying the point of application of the stimulation from day to day. The forward component of the excursion of the foot is used as the index of the knee-jerk and is obtained by attaching the heel of the subject's shoe by a strong, inelastic cord to a stylus which writes on a smoked drum. The distance through which the stylus moves when the stimulus is delivered is referred to as the height of the knee-jerk.

Table I shows a comparison of the average height of the knee-jerks of each subject for each period during the menstrual cycle.

TABLE I

PERIOD	AVERAGE HEIGHT OF KNEE-JERK SUBJECT NO.				
	1	2	3	4	5
1	27.00	19.40	22.50	23.00	6.16
2	28.60	18.00	19.75	27.00	16.00
3	29.00	18.85	25.01	17.60	14.00
4	30.00	28.75	16.50	8.10	20.02

Subject 1 is an instructor in this department and is thirty years of age. She reports that ordinarily menstruation causes her no unusual inconvenience. Her records were made at 11:20 A.M. As shown by Table I there are no significant changes in the extent of the knee-jerk during the menstrual cycle.

Subject 2 is an instructor in the university and is twenty-three years of age. She reports that menstruation does not alter her physical condition as far as she is able to observe. The data presented in Table I were collected at 1:00 P.M. They indicate a hypoirritable condition during Periods 1, 2, and 3, followed by increased irritability during Period 4.

Subject 3 is a student twenty-five years of age. Her records were made at 8:00 A.M. The data in Table I indicate that Period 2 is preceded and followed by periods of hyperirritability. The subject is less irritable during Period 4. This subject volunteered no information concerning her observations with reference to the effect of menstruation on herself.

Subject 4 is twenty-four years of age and is an instructor in the university. Her records were made at 5:00 P.M. The data show that in her case there is a tendency toward increased irritability which reaches its maximum

during Period 2, gradually returning to normal during Periods 3 and 4. This subject reported that she felt more irritable during Period 2 than otherwise.

Subject 5 is an assistant in the university and is thirty-eight years of age. The data show that irritability is greatest during Period 4. Period 2 is preceded by a period of marked depression and is followed by a slight decrease in irritability.

Care was taken that the habits and activities of the subjects were as nearly uniform as possible for each day. Precautions were taken to prevent the operation of any of the significant factors which have been shown to affect the knee-jerk.

In observing the various subjects the writer noticed that, with but little exception, the records showed that disturbing factors had much more marked effect during Periods 1, 2, and 3 than during Period 4. This observation we believe is the most important point brought out in this investigation.

The data herein presented seem to indicate that the condition of the individual subject is a determining factor in the effect of menstruation. Some women will, no doubt, exhibit marked periods of increased and decreased irritability during the cycle in all cases; some will show this variation at different times, while others will show little or no effect. The investigations, up to the present time hardly justify any generalization with reference to the variation in irritability during the menstrual cycle.

The data presented in this paper, based on a very limited group of women, so far as they go show that: (*a*) there is, in some cases, no significant change of irritability during the menstrual cycle; (*b*) there may be a condition of depressed irritability during and preceding the menstrual period; (*c*) there may be a condition of hyperirritability preceding, during, and following the menstrual period. These findings support the conclusion, borne out by the general trend of recent investigations, that in case of healthy women, menstruation leads to no very significant disturbance of functions. Such variations as are noted are inconstant and inconsistent.

SUMMARY

While studying the change of irritability of five women during the menstrual cycle it was found that in one case there was no significant change in irritability; in one case, during Periods 2 and 3 there was a condition of depression; in one case there was a condition of hyperirritability during Periods 1 and 3; in one subject there was a condition of hyperirritability developed during Period 1, reaching a maximum during Period 2 and then gradually returning to normal during Periods 3 and 4; in one subject there was a period of marked depression during Period 1, followed by increased irritability in Period 2, gradually reaching a maximum during Periods 3 and 4. The data further show that any disturbing influences affecting the knee-jerk have a more marked influence during Periods 1, 2, and 3.

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REPORT OF A VERY SEVERE CASE OF JUVENILE DIABETIC COMA IN WHICH COMBINED TREATMENT WITH INSULIN AND BLOOD TRANSFUSIONS RESULTED IN PROMPT RECOVERY*

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BLOOD transfusions without insulin have been recorded in the treatment of diabetic coma, but the results have been mostly unfavorable. The justification for investigating the influence of transfusion upon the insulin-treated patient was furnished by the case of a moribund boy in profound dyspneic coma, who had failed to react favorably to the administration of large doses of insulin. Blood transfusion was then employed as a last resort. There was an instantaneous and very definite turn for the better, and the patient walked out of the hospital on the sixth day.

REPORT OF A CASE

The patient, a Jewish boy ten years old, was admitted in a state of coma to the United Israel-Zion Hospital, Sept. 18, 1924. The family history was negative. The patient had had measles, whooping cough, bronchopneumonia, tonsillitis and chorea. The tonsils and adenoids had been removed three years previously. The present illness began in the summer of 1923 with gradual loss of strength and weight and pain in the epigastrium. A physician found 5 per cent of sugar in the urine and a blood-sugar level of 170 mg. per 100 c.c. The boy was treated dietetically and with insulin, and the urine became sugar-free. In February, 1924, when insulin was discontinued for a time, the blood sugar promptly rose to 640 mg. per 100 c.c., and the urine contained 6 per cent of sugar. Treatment with insulin was resumed (8 to 10 units t. i. d.) and the blood sugar became normal and the urine sugar-free.

Unfortunately the boy fell into the hands of a quack, upon whose advice all insulin was discontinued on Sept. 16, 1924, and a liberal diet was allowed. On the following evening, he began to vomit and complained of great thirst and pain in the precordium and epigastrium. On Sept. 18, the patient passed into a comatose state and was hurried to the hospital.

Examination disclosed a sallow, poorly nourished boy, weighing 58 pounds, in deep coma. The temperature was 99.4° F., pulse 120, respiration 30. Breathing was of the Kussmaul type. The body and extremities were cold and clammy to the touch, the lips were cyanosed, and the alae nasi were widely dilated. There was a strong odor of acetone to the breath. The tongue was extremely dry and rough. The knee-jerks were absent. The skin of the extremities showed numerous needle points from previous injections of insulin and scars from old boils.

On admission, the urine had a specific gravity of 1028, and contained 3.3 per cent of sugar and a very large amount of acetone bodies. The blood sugar was 666 mg. per 100 c.c. The carbon dioxide combining power of the blood plasma, as measured by the method of Van Slyke and Cullen, was down to the extremely low figure of 7 volumes per cent. The red blood cell count was 3,600,000, hemoglobin 67 per cent (Sahli corrected). The white blood cells numbered 20,000, with 89 per cent polynuclears and 11 per cent lymphocytes.

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The boy entered the hospital at noon, and at 12:15 P.M. he received 35 units of insulin (H-insulin Lilly). At 12:30 P.M. a hypodermoclysis of 1,000 c.c. of salt solution was administered. At 2:30 P.M. 40 units of insulin were given, and at three o'clock 35 more units. The child came out of his coma, but would only open his eyes when disturbed and pushed.

At 3:30 P.M. 250 c.c. of blood were transfused from a healthy compatible donor by a direct vein-to-vein method. Within five minutes of this operation, there was a spectacular turn for the better. The patient became brighter, his eyes remained open, and he asked for water. The cyanosis disappeared, a good color returned to the cheeks, the pulse became stronger, and the respirations began to approach the normal type.

At four o'clock a hypodermoclysis of 800 c.c. of saline solution was administered. Thirty-five units of insulin were injected at 7:30 P.M., but the patient again appeared drowsy and the pulse was rapid and irregular. At 9:30 P.M. a second blood transfusion was done, injecting 250 c.c. of blood, and, as in the first instance, there was an immediate favorable response. The child brightened up and asked for water, and the pulse became stronger and more regular. Orange juice and water were allowed. Twenty-five units more of insulin were given at eleven o'clock. The patient was now out of coma and would answer when spoken to. When observed at 1:30 A.M., the boy was perfectly comfortable. His mental condition was clear and he asked for food and water.

At 2:30 A.M. the child complained of headache, and soon thereafter he began to sweat profusely. Symptoms apparently attributable to insulin shock were noted at 3:30 A.M. The boy suddenly awoke and became restless, constantly moaning. The pulse was of poor quality and respiration became of the Cheyne-Stokes' type. An intramuscular injection of 1 c.c. of adrenalin was given at 4:30 A.M., but breathing became deep and stertorous, and the patient began to foam at the mouth. Seven hundred cubic centimeters of saline solution were injected by hypodermoclysis, and at 6:30 A.M. the adrenalin was repeated. Oxygen was administered. The child rallied and by morning appeared very bright. On Sept. 19, at 9:30 A.M. the blood sugar was 110 mg. per 100 c.c. and the carbon dioxide combining power of the blood plasma was 50 volumes per cent.

From this time on improvement was rapid and progressive, and the patient was discharged from the hospital on Sept. 23, only five days from the time when he had been admitted in absolute coma. While in the hospital, he received 10 units of insulin three times a day, and the diet had a value of 15 gm. of carbohydrate, 25 gm. of protein, and 35 gm. of fat. At the time of discharge, the boy felt perfectly well, his urine was sugar-free, and his blood sugar was 111 mg. per 100 c.c.

When last examined on Dec. 1, 1924, the patient had no complaint, had not missed a day at school since Oct. 15, and played like a normal boy. He had gained 3 pounds, his general appearance, color, and muscle tone were good, and he was active and alert. The urine was not sugar-free but contained from 4 to 7 gm. of glucose in the twenty-four-hour specimens. Occasionally there was a trace of acetone and diacetic acid. The patient was on a diet of 60 gm. of carbohydrate, 65 gm. of protein, and 70 gm. of fat. He was receiving 40 units of insulin a day, 20 units before breakfast and 20 units before the evening meal. It was not deemed desirable to attempt to render the urine sugar-free for two reasons: (1) To accomplish this end by still further reduction of the diet seemed unwise in view of the state of the patient's nutrition; and (2) the fact that on several occasions there had been manifestations of insulin shock necessitated great care in adjusting the dosage of this agent.

COMMENT

Blood transfusion was tried in the treatment of diabetic coma before the days of insulin, but the results were mostly unfavorable. It has generally been held that the presence of diabetes mellitus is a contraindication of this procedure. Raulston and Woodyatt,[1] in 1914, reported a severe case of diabetic acidosis, in which the transfusion of 500 c.c. of peripheral venous blood from a healthy male donor had a decided effect for the worse on the

metabolism of the patient, as evidenced by a marked rise in the output of sugar, ammonium and acetone bodies and an increase in the glucose: nitrogen ratio. The patient died in typical dyspneic coma. Ottenberg and Libman,[2] in 1915, employed blood transfusion in four severe cases of diabetes mellitus, but failed to observe any benefit from this procedure. Three of the patients were in diabetic coma, while the fourth patient was a young woman with both diabetes mellitus and pernicious anemia. In no case did the transfusion have more than a transient effect on the coma. Garbat[3] treated a patient with diabetic coma by means of venesection and the removal of 500 c.c. of blood followed by blood transfusion by the citrate method. The coma had been of sudden onset following an operation for gangrene of the foot. There was distinct temporary improvement; the patient revived from her coma, but relaxed again in twenty-four hours. Venesection and transfusion were repeated, but, while a noticeable improvement again occurred, the patient did not recover.

Animal experiments have offered greater encouragement as to the efficacy of blood transfusion in diabetes mellitus than have the actual clinical trials. Carlson and Ginsburg,[4] in 1915, transfused dogs rendered diabetic by pancreatectomy with blood from normal dogs. The amount of blood transfused was approximately one-tenth that of the volume of the recipient's blood. As a result of this procedure, these workers observed a temporary (lasting four to eight hours) but definite and consistent lowering of the hyperglycemia and glycosuria. When diabetic dogs were used as donors, there was no effect on the hyperglycemia.

Notwithstanding this lack of encouragement from the literature, it was felt that the greatly enhanced metabolic capacity conferred by the administration of large doses of insulin might enable the diabetic to utilize an increment of fresh blood and thus to increase the level of oxidation, relieve dehydration, and enrich the buffer value of the blood. In a suboxidative disease such as diabetic coma, one would certainly expect a substantial addition of oxygen-carriers to be a potent factor leading to recovery. Acting upon the assumption that the earlier reported unfavorable results from blood transfusion in diabetes mellitus were largely attributable to the incapacity of the metabolism to handle the added burden of the transfused blood, and believing that the great aid to carbohydrate combustion rendered by the administration of insulin would remove this danger, we decided to try combined insulin therapy and blood transfusion as a last resort in this extremely severe case of diabetic coma.

Of course, it is realized that conclusions as to the efficacy of any therapeutic procedure based on the result obtained in a single case are not justified, but in this instance, the favorable response to blood transfusion in the insulin-treated patient was so instantaneous and definite as to strongly suggest that the transfusion played an important part in the patient's recovery. Any attempt in the present case to distinguish between the good which resulted from the insulin and that which could be attributed to the blood transfusion would be impossible.

SUMMARY

In the case of a comatose boy, aged ten years, with clinical and laboratory evidences of severe diabetes mellitus and maximal acidosis, large doses of insulin did not appear to yield marked benefit. A transfusion of 250 c.c. of unmodified blood in this insulin-treated patient, however, resulted in instantaneous and very definite improvement and the boy immediately came out of the coma. A second similar transfusion six hours later was again followed by striking betterment, and the patient walked out of the hospital on the sixth day. Although reports show that blood transfusions in diabetic patients without insulin have yielded mostly unfavorable results, the striking benefit noted in the present case is strongly suggestive of the possibility that blood transfusion in conjunction with large doses of insulin may prove to be a very valuable procedure in the treatment of diabetic coma.

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LABORATORY METHODS

A MICRO-FOLIN-WU METHOD OF QUANTITATIVE BLOOD-SUGAR ESTIMATION, USING 0.1 C.C. OF BLOOD*

By T. L. Byrd, M.D., Milwaukee, Wisconsin

THE present-day methods available for collecting blood specimens for sugar determinations are not applicable to all patients, and any suggestion of improvement over methods now in use will be welcomed by physicians and patients, where simplicity and accuracy are the ultimate ends.

It is obvious to all medical practitioners that a simple, accurate micromethod of quantitative blood-sugar estimation is needed; one which requires a minimum quantity of blood that can be obtained from finger or ear by a prick of a pin, under aseptic precaution; one which eliminates the fear, pain, and danger of a venepuncture, and saves time and effort for the operator; a method applicable to infants, small children, and obese individuals, from whom it is almost an impossibility to obtain blood for analyses, for reasons well known. With the unlimited use of insulin, a method with these advantages is necessary.

Within the past few years several attempts have been made, and micro modifications of the Folin-Wu method have been devised and published by Pollock and McEllroy,[1] Haden,[2] Baum and Isaacson,[3] Kramer and Gittleman,[4] Randles and Grigg,[5] and a number of micro-Lewis-Benedict methods. All micromethods heretofore described are modifications of the original Folin-Wu method, and necessitate variations in technic, which render them impracticable from a standpoint of simplicity and accuracy, for practical routine use.

The Folin-Wu method of analysis is one of the best now in use; when 1 volume of blood and 7 volumes of distilled water are mixed, complete hemolysis occurs; 0.1 c.c. of blood, plus 0.7 c c. of distilled water plus 0.1 c.c. of 10 per cent sodium tungstate plus 0.1 c.c. of two-thirds normal sulphuric acid, precipitated and centrifuged at high speed for five to ten minutes, yields 0.7 c.c. of clear blood filtrate. Analysis of 0.5 c.c. and 2 c.c. of blood filtrate under similar conditions using solutions in proportion, will give the same end-results.

Evidently a device for collecting, measuring, and diluting 0.1 c.c. of blood, applicable to all patients is needed to perfect a micromethod of blood-sugar estimation. The idea was conceived of a blood diluting pipette, graduated at 0.1 c.c. and 0.8 c.c. respectively, with two chambers, the first having a capillary intake expanding into a fusiform shape of 0.1 c.c. capacity for obtaining and measuring the blood. The second having 0.7 c.c. capacity for

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diluting, mixing, and hemolyzing, with a constriction of the bore at each graduation. It is thus constructed to prevent the blood from flowing out, in case the operator has to pause to express more blood from the bleeding point; to insure accurate measurements; and so it can be held between the finger and thumb for mixing. The pipette is supplied with a detachable rubber tube and mouthpiece, and used in the same manner in obtaining and diluting blood as one for blood counting. This pipette solves the problem of obtaining blood from patients in small quantity. It is applicable to all patients, including infants, small children, and obese individuals. To those physicians in general practice, who have to collect blood and transport it to a laboratory, this instrument will be very useful, for the reason that a rubber band placed over the ends of the pipette after the blood is drawn and diluted, will enable it to be carried any distance without loss, and it will keep in this manner

TABLE I

TIME BLOOD WILL REMAIN PRESERVED AT ROOM AND INCUBATOR TEMPERATURE IN THE PIPETTE WITH A RUBBER BAND PLACED OVER THE ENDS

DILUTED WITH DISTILLED WATER

Blood No. 1 when taken 400 mg. after standing 9 hr. room temp. 400 mg.
Blood No. 2 when taken 400 mg. after standing 12 hr. room temp. 333.3 mg.
Blood No. 3 when taken 100 mg. after standing 24 hr. room temp. 0 mg.

DILUTED WITH 1:400 FORMALDEHYDE SOLUTION

Blood No. 1 when taken 285.7 mg. after standing 168 hr. (7 days) incub. 37.5° C. 285.7 mg.
Blood No. 2 when taken 250 mg. after standing 288 hr. (12 days) rm. temp. 250 mg.

TABLE II

ANALYSES OF 22 SPECIMENS OF BLOOD OF NONDIABETIC PATIENTS BY THE ORIGINAL AND THE MICRO-FOLIN-WU METHODS OF BLOOD-SUGAR ESTIMATION

ORIGINAL FOLIN-WU METHOD USING 2 C.C. OF BLOOD, OBTAINED BY VENEPUNCTURE			AUTHOR'S MICRO FOLIN-WU METHOD USING 0.1 C.C. OF BLOOD, OBTAINED WITH BLOOD DILUTING PIPETTE
No.	Name	Mg. per 100 c.c. of Blood	Mg. per 100 c.c. of Blood
1.	Mrs. I. R.	100	100
2.	Mrs. T. J.	100	100
3.	Mrs. E. M.	100	100
4.	Mr. O. R. T.	100	100
5.	Mrs. H. M.	100	100
6.	Mrs. J. H. M.	111	111
7.	Miss I. F.	111	111
8.	Mrs. W. B. B.	100	100
9.	Mrs. P. T. S.	100	100
10.	Mr. Wm. J.	100	100
11.	Mrs. B. B.	90.9	90.9
12.	Mrs. K. Z.	100	100
13.	Mrs. K. J.	90.9	90.9
14.	Mrs. A. C.	100	100
15.	Mrs. S. M.	100	100
16.	Mr. F. P. G.	83.3	83.3
17.	Mrs. M. S.	100	100
18.	Mrs. K. H.	100	100
19.	Mrs. P. C. M.	100	100
20.	Mr. J. L. Mc.	90.9	90.9
21.	Mrs. M. B.	111	111
22.	Mrs. K. S.	111	111

NOTE.—The colorimeter was set at the Number 10, the unknown of one technic was read; the standards changed, and the unknown of the other technic brought to the same figure as the former. (Whole numbers were used.)

from nine to twelve hours. In case it is necessary to send by mail, it can be diluted with 1:400 formaldehyde solution, instead of distilled water, which will preserve the blood for one week or more, at incubator (37.5° C.) or room temperature, without altering the sugar content. The Folin sugar tubes were reduced to one-fourth size, and other apparatus needed was supplied, thus minimizing, and not modifying the orginal technic in any single detail. A preliminary report with a drawing of the apparatus needed for the micro-Folin-Wu method, was published in the *Journal of the American Medical Association*, August 16, 1924.

Analyses were made on 86 specimens of blood under similar conditions, by the original and the micro-Folin-Wu method, without a single discrepancy. Tables II and III give the results obtained.

TABLE III

ANALYSES OF 28 SPECIMENS OF BLOOD OF DIABETIC PATIENTS BY ORIGINAL AND THE MICRO-FOLIN-WU METHOD OF BLOOD-SUGAR ESTIMATION

ORIGINAL FOLIN-WU METHOD USING 2 C.C. OF BLOOD, OBTAINED BY VENEPUNCTURE			AUTHOR'S MICRO FOLIN-WU METHOD USING 0.1 C.C. OF BLOOD, OBTAINED WITH BLOOD DILUTING PIPETTE
No.	Name	Mg. per 100 c.c. of Blood	Mg. per 100 c.c. of Blood
1.	Mr. J. R. F.	200 (a)	200 (a)
2.	Mr. J. R. F.	153.8(b)	153.8(b)
3.	Mr. J. T. M.	333.3(a)	333.3(a)
4.	Mr. J. T. M.	500 (b)	500 (b)
5.	Mrs. T. A. O.	181.8	181.8
6.	Mrs. McK.	133.3	133.3
7.	Mrs. P. A.	200 (a)	200 (a)
8.	Mrs. P. A.	142.8(b)	142.8(b)
9.	Mr. S. V. H.	125	125
10.	Miss M. D.	333.3	333.3
11.	Sr. M. M.	142.8	142.8
12.	Mrs. A. K.	400 (a)	400 (a)
13.	Mrs. A. K.	200 (b)	200 (b)
14.	Mr. G. B.	166.6	166.6
15.	Mr. L. P. K.	125	125
16.	Mrs. E. J.	142.8	142.8
17.	Mrs. S. J.	333.3	333.3
18.	Mr. J. E. P.	181.8	500
19.	Mr. H. F. D.	500	500
20.	Mr. F. R. F.	125.9	125.9
21.	Mr. T. A. C.	125	125
22.	Mrs. A. O. B.	142.8	142.8
23.	Miss H. A.	125	125
24.	Miss L. B.	125	125
25.	Mrs. C. P.	250	250
26.	Miss R. B.	250	250
27.	Miss E. S.	222.2	222.2
28.	Mrs. S. J.	200	200

NOTE.—All bloods were drawn and analyzed under the same conditions.

The data obtained from the sugar tolerance test is very valuable in cases of mild diabetes, renal diabetes, obesity, and pituitary disturbances. It is seldom done because it subjects the patient to several venepunctures at one-half and one hour intervals. This method removes every obstacle. Six tests were done using six specimens each, making a total of thirty-six specimens. Chart 1 gives the results obtained.

AUTHOR'S MICRO-FOLIN-WU METHOD

I. Method of obtaining blood from patient.

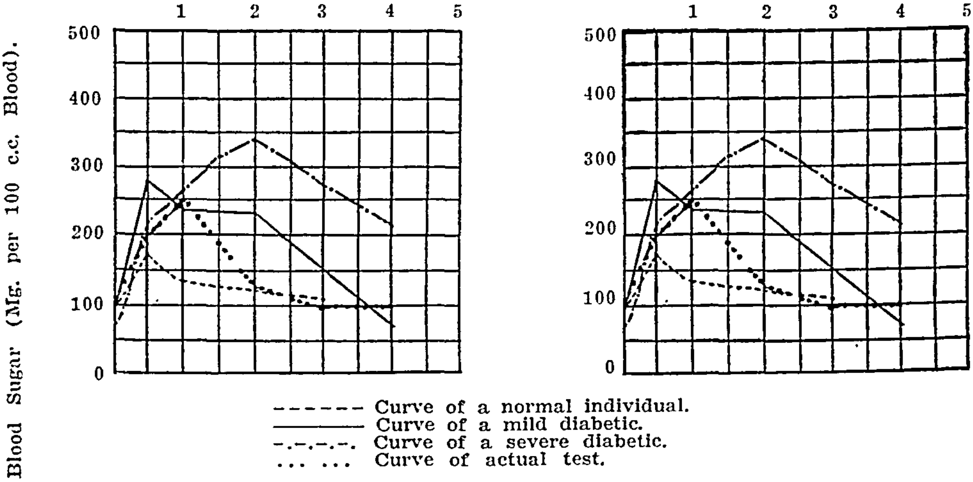
1. Rinse special blood diluting pipette with distilled water, alcohol, and ether; draw potassium oxalate solution, (5 to 10 per cent) to just above the 0.1 c.c. mark (expel the excess) to prevent the blood from adhering to the sides of the lower chamber of the pipette. (Where a number of bloods are to be taken with a single pipette, washing with distilled water, and the potassium oxalate solution will suffice as distilled water is the diluting fluid.)

2. Clean finger tip or ear with alcohol or iodine, prick with pin point or lancet, cleaned with the same drug, and from bleeding point, by means of blood diluting pipette, draw blood to 0.1 c.c. mark; wipe tip with a cloth or cotton, then emerse in distilled water and draw to 0.8 c.c. mark; shake well; complete hemolysis results.

CHART I

Folin-Wu Method — Micro-Folin-Wu Method

Time in hours after ingestion of glucose.



------ Curve of a normal individual.
———— Curve of a mild diabetic.
-.-.-.-. Curve of a severe diabetic.
... ... Curve of actual test.

Grams glucose given by mouth ....100..............

Blood Sugar per 100 c.c.			Urine sugar per cent	
Fasting	100.0	Mg.	Negative	%
30 Min.	200.0	"	Negative	%
60 Min.	250.0	"	Slight reduction	%
2 Hr.	133.3	"	Slight reduction	%
3 Hr.	100.0	"	Negative	%
4 Hr.	100.0	"	Negative	%

Remarks .... Normal Curve.

II. Precipitation of blood.

1. Transfer contents of pipette, (1 vol. of blood plus 7 vol. of distilled water) to a pyrex centrifuge tube.

2. Add 0.1 c.c. (1 vol.) of 10 per cent sodium tungstate solution; stir with glass rod.

3. Add 0.1 c.c. (1 vol.) of two-thirds normal sulphuric acid solution; stir with glass rod; thus precipitated, let stand from ten to twenty minutes.

III. Centrifuge at high speed from five to ten minutes. This yields 0.5 to 0.7 c.c. of clear filtrate.

IV. *Method of sugar estimation.*

1. Place 0.5 c.c. of blood filtrate in a minimized Folin sugar tube, graduated at 1 c.c. bulb and 6¼ c.c. total measurement.

2. Place 0.5 c.c. of sugar, standard solution, containing 0.2 mg. and 0.4 mg. per 2 c.c. respectively, in two other similar tubes.

3. Add 0.5 c.c. of alkaline copper solution to each tube.

4. Place in a water-bath and boil for six minutes.
5. Transfer to cold water-bath for from two to three minutes.
6. Add 0.5 c.c. of molybdate phosphate solution to each tube and let stand two minutes.
7. Add distilled water to 6¼ c.c. mark on each tube and compare in a colorimeter, the unknown to the standard, nearer comparing to the naked eye.
8. Calculation:

$$\text{(Weaker)}\quad \frac{S}{U} \times 100 = \text{mg. per 100 c.c. of blood.}$$

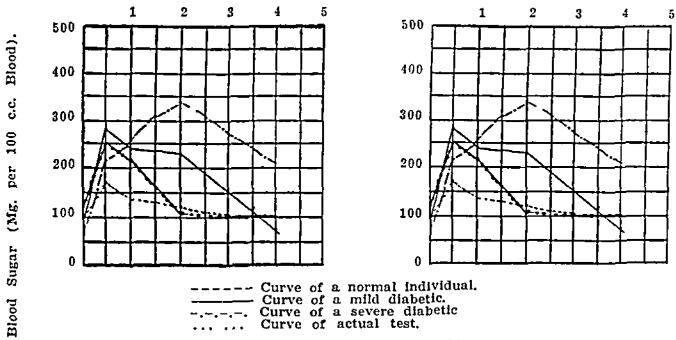
$$\text{(Stronger)}\quad \frac{S}{U} \times 200 = \text{mg. sugar per 100 c.c. of blood.}$$

NOTE.—The apparatus is washed with water. The pipettes are rinsed with the solution to be added, to avoid diluting.

CHART II

Folin-Wu Method — Micro-Folin-Wu Method

Time in hours after ingestion of glucose.



Grams glucose given by mouth ....100..............

Blood Sugar per 100 c.c.		Urine sugar per cent	
Fasting	125.0 Mg.	Negative	%
30 Min.	250.0 "	1.16	%
60 Min.	222.2 "	2.27	%
2 Hr.	111.0 "	0.65	%
3 Hr.	100.0 "	Negative	%
4 Hr.	100.0 "	Negative	%

Remarks .... Renal diabetic.

PREPARATION OF SOLUTION

The solutions used are prepared the same as for the original, and in the following manner:

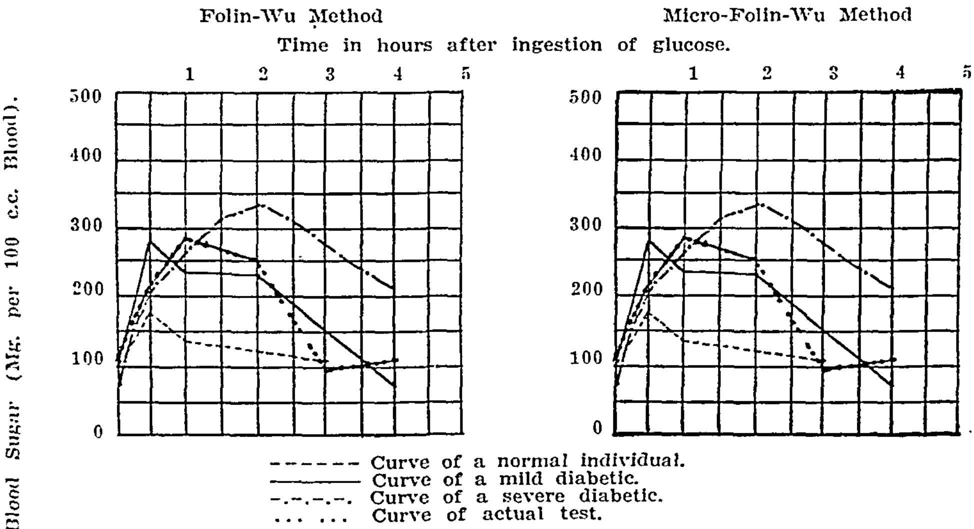
The standard sugar solution is prepared by dissolving 1 gm. pure anhydrous dextrose in 100 c.c. of saturated solution of benzoic acid. The working solutions are prepared by diluting 5 and 10 c.c. of the above stock solution to 500 c.c. with saturated solution of benzoic acid. These will contain 0.2 and 0.4 mg. dextrose per 2 c.c., respectively, and are fairly stable.

The alkaline copper solution is prepared by dissolving 40 gm. anhydrous

Na_2CO_3 in about 400 c.c. distilled water and placing in a liter flask. Add 7.5 gm. tartaric acid and when dissolved add 4.5 gm. crystallized $CuSO_4$; mix and make up to 1 liter. With impure carbonate, a sediment may form in a week or so; in such case, filter into another vessel.

The molydbate phosphate solution is prepared by putting 35 gm. molybdic acid in a one liter beaker. Add 5 gm. sodium tungstate, 200 c.c. 10 per cent NaOH, and 200 c.c. distilled water. Boil vigorously from twenty to forty minutes to remove NH_3; cool and dilute to 350 c.c. with distilled water. Add 125 c.c. 85 per cent phosphoric acid and dilute to 500 c.c. with distilled water.

CHART III



Grams glucose given by mouth ....100..............

Blood Sugar per 100 c.c.			Urine per cent	
Fasting	100.0	Mg.	Negative	%
30 Min.	222.2	"	1.25	%
60 Min.	285.7	"	3.57	%
2 Hr.	250.0	"	3.12	%
3 Hr.	99.9	"	Slight reduction	%
4 Hr.	111.1	"	Negative	%

Remarks ....Mild diabetic.

On account of the simplicity, accuracy, and many advantages of the micro-Folin-Wu method, it has been adopted as routine in our institution. The members of the staff order blood-sugar and sugar-tolerance tests on patients in the same manner as ordering a blood count, and all specimens are taken and transported to the laboratory by the technicians, in the same way, for the technic is the same. Up to the present time 100 specimens of blood have been analyzed and Tables IV and V will give results in a number of these cases.

CONCLUSIONS

This micromethod is a minimized and not a modified Folin-Wu method of blood-sugar estimation. It does not vary in a single detail from the original technic.

TABLE IV

ANALYSES OF 24 SPECIMENS OF BLOOD OF NONDIABETIC PATIENTS BY AUTHOR'S MICRO-FOLIN-WU METHOD, USING 0.1 C.C. OF BLOOD

NO.	NAME	MG. PER 100 C.C. OF BLOOD
1.	Dr. T. L. B.	90.9
2.	Miss M. F.	100
3.	Miss V. G.	100
4.	Mrs. J. D.	100
5.	Miss J. S.	100
6.	Miss H.	100
7.	Miss M. H.	100
8.	Miss E. C.	100
9.	Miss H. B	100
10.	Mr. L. F. D.	83.3
11.	Miss L. A. E.	100
12.	Miss S. S	90.9
13.	Mrs. J. McD.	100
14.	Mrs. J. O. C.	100
15.	Mrs. G. E. E.	100
16.	Mrs. S. G.	100
17.	Mrs. M. C. O	100
18.	Mr. L. F.	90.9
19.	Miss W. W.	100
20.	Miss W. A.	100
21.	Miss K. D.	100
22.	Miss G. E.	100
23.	Miss W. W.	100
24.	Miss K. K.	90.9

NOTE.—Read on whole numbers.

TABLE V

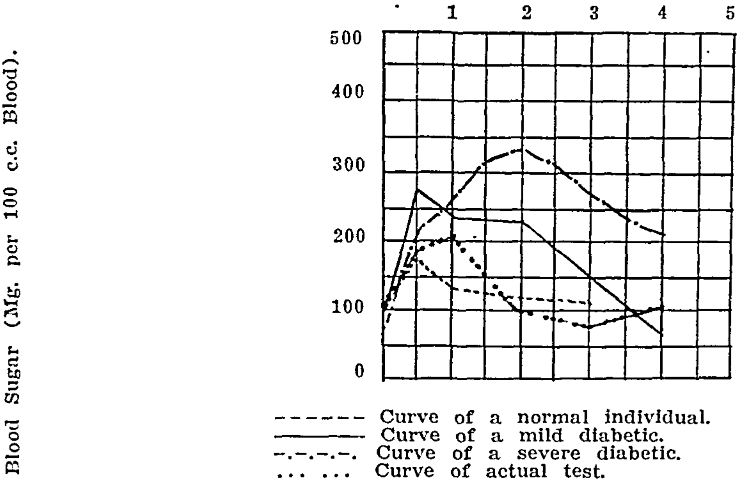
ANALYSES OF 26 SPECIMENS OF BLOOD OF DIABETIC PATIENTS BY AUTHOR'S MICRO-FOLIN-WU METHOD, USING 0.1 C.C. OF BLOOD

NO.	NAME		MG. PER 100 C.C. OF BLOOD
1.	Mrs. M. H.		333.3
2.	Mrs. S. S.		333.3
3.	Mrs. C. G.		400
4.	Mr. J. S.		200
5.	Mrs. A. S.		333.3
6.	Mr. J. K.		200
7.	Sr. M. B.		500 (a)
8.	Sr. M. B.		400 (a)
9.	Mrs. M. D.		400 (a)
10.	Mrs. M. D.		400 (b)
11.	Mrs. M. D.		400 (c)
12.	Mrs. M. D.		285.7(d)
13.	Mrs. M. D.		285.7(e)
14.	Mrs. M. D.	Insulin Treatment	333.3(f)
15.	Mrs. M. D.		400 (g)
16.	Mrs. M. D.		333.3(h)
17.	Mrs. M. D.		285.7(i)
18.	Mrs. M. D.		181.8(j)
19.	Mrs. M. D.		166.6(k)
20.	Mrs. M. D.		142.8(l)
21.	Mrs. M. D.		133.3(m)
22.	Mrs. M. D.		166.6(n)
23.	Mrs. M. D.		142.8(o)
24.	Mrs. M. D.		153.8(p)
25.	Mrs. M. D.		111. (q)
26.	Mrs. M. D.		133.9(r)

CHART IV

Micro-Folin-Wu Method

Time in hours after ingestion of glucose.



------ Curve of a normal individual.
——— Curve of a mild diabetic.
–.–.–.–. Curve of a severe diabetic.
... ... Curve of actual test.

Grams glucose given by mouth..........100..............

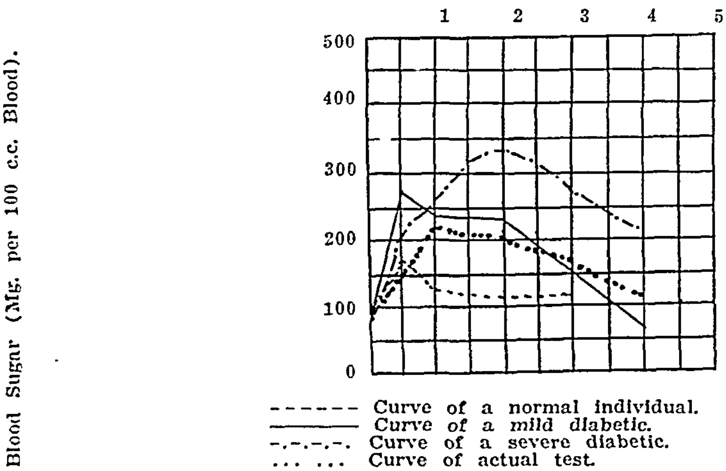
Blood sugar per 100 c.c.			Urine sugar per cent	
Fasting	100.0	Mg.	Negative	%
30 Min.	181.8	"	0.4	%
60 Min.	222.2	"	1.85	%
2 Hr.	100.0	"	1.35	%
3 Hr.	83.3	"	Slight reduction	%
4 Hr.	90.9	"	Negative	%

Remarks .... Renal diabetic.

CHART V

Micro-Folin-Wu Method

Time in hours after ingestion of glucose.



------ Curve of a normal individual.
——— Curve of a mild diabetic.
–.–.–.–. Curve of a severe diabetic.
... ... Curve of actual test.

Grams glucose given by mouth..........100..............

Blood sugar per 100 c.c.			Urine sugar per cent	
Fasting	100	Mg.	Negative	%
30 Min.	133.3	"	Slight reduction	%
60 Min.	222.2	"	1.56	%
2 Hr.	200	"	1.92	%
3 Hr.	166.6	"	1.72	%
4 Hr.	125	"	0.56	%

Remarks .... Mild diabetic.

The blood diluting pipette solves the problem of obtaining the blood in small quantities, and is applicable to all patients, including infants, small children, and obese individuals, in whom it is almost impossible to obtain blood by venepuncture. There is no danger of blood clotting in the pipette, because there is ample time to draw and dilute the blood, and this can be done by any technician who can obtain blood for counting, for the technic is the same; while venepuncture usually requires the skill of a physician. This pipette provides a simple means whereby physicians out in general practice can obtain, measure, and dilute specimens of blood and transport them to a laboratory, by placing a rubber band over the ends. Diluted with distilled water, it will keep from nine to twelve hours at room temperature. Using 1:400 formaldehyde solution as diluent, it will remain preserved for one week or more, at incubator (37.5° C.) or room temperature, without altering the sugar content in the least. This makes it possible to send specimens of blood in this manner any distance through the mail.

This method has distinct advantages over the original, because it eliminates the necessity of having on hand sterile syringes and needles, and test tubes containing potassium oxalate.

It saves the patient the fear, pain, and danger of venepuncture.

It saves time for the operator, since blood is drawn, measured and diluted in a few seconds' time.

Its simplicity and accuracy make it desirable for routine use in any institution, clinic, or private laboratory, and for these reasons I submit it to the profession, since in all scientific investigation, we should endeavor to subject our patients to as little pain as possible, and at the same time give accurate results.

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A SIMPLE AND SENSITIVE MODIFICATION OF THE WASSERMANN TEST*

By E. E. Ecker, Ph.D., Cleveland, Ohio

INTRODUCTION

SINCE the Wassermann test has become a widely employed laboratory procedure many attempts have been made to simplify the method, to render it more sensitive and accurate. As with any other laboratory procedure, familiarity with a given method produces a degree of confidence that is sometimes unjustified by the facts. Many attempts have been made in various countries to devise a standard method which can be adopted by a large number of laboratories. Throughout this country the labors of Kolmer[1] and his associates are well and favorably known and the modification they have proposed has been widely adopted. Our studies of the situation are in general agreement with those of Ottenberg[2] and Ottenberg and Wisler,[3] who find that the Kolmer modification is somewhat cumbersome, employs larger amounts of material than seems absolutly essential and fails to make provision for that antisheep amboceptor not uncommonly present in human serum. This is further complicated by the fact that the methods so far devised for eliminating the antisheep amboceptor in human serum are likely to increase the anticomplementary activity of that serum. It is difficult to compel all laboratories to adopt a uniform method, and admitting that this aim is desirable, widespread adoption should depend rather upon convincing evidence that a given method is the best so far available than by setting up a standard upon any particular group of appointed individuals. It is hoped that the method herein described will, with proper indication and control, meet the necessities of the present unfavorable situation. Its principle advantages are simplicity, avoidance of the interfering reaction due to the presence of antisheep amboceptor in human serum, and what is believed to be increased sensitivity without particular danger of false positives. The method employs a uniformly prepared antigen, five doses of hemolytic amboceptor and delicately titrated complement. It is well known that the phenomenon of hemolysis will occur in the presence of one unit of cell suspension, one unit of amboceptor and one dose of complement. It is equally well known that if the dose of amboceptor be increased, the dose of complement can be reduced. Furthermore, if a test of this sort be set up with four units of hemolytic amboceptor, one dose of cell suspension and a carefully titrated amount of complement, the addition of one or two units of amboceptor derived either from a specific immune serum or from human serum, the outcome is not demonstrably altered. These are the principles underlying the proposed

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modification which in seven years of practice, has given convincing evidence of simplicity, sensitivity and accuracy.

In the report of the hygienic committee of the League of Nations given by Sachs,[4] it is pointed out that there is no advantage in employing more than one antigen in these tests. The method of preparation of antigen given in this paper has been constantly successful. It has a high titer with low anticomplementary and no hemolytic activity. It is an acetone insoluble antigen and for routine work requires no cholesterol or other substance to increase its binding power.

The method of titration of complement should be most carefully followed. Ice box fixation has proved to be of the utmost value. As the result of numerous tests with this method it has been found, as pointed out by Kline, Applebaum and Lundagen,[5] that overnight ice box fixation is distinctly preferable to four hour fixation, because it increases the sensitiveness of the test and permits the recognition of partial fixation in treated and doubtful cases. The principles embodied in this communication have been followed in numerous hospitals in the city of Cleveland with the utmost satisfaction.

PREPARATION OF MATERIALS

Antigen.—The antigen as now employed is a modification of Ecker and Sasano's antigen.[6] The Bordet and Ruelens[7] antigen may also be used with success. The new antigen as prepared by the author is as follows: Extract in a 500 c.c. Erlenmeyer flask, with attached coil condenser over a water-bath, 30 grams of finely powdered, rapidly dried, mixed calf or beef hearts with 200 to 300 c.c. of C. P. acetone for a period of three to four hours. Avoid overheating. Remove from water-bath and chill mixture in ice box overnight. Filter through paper and allow acetone to evaporate entirely in an incubator at 37° C. Extract the acetone-free dry powder in the same apparatus with 200 to 300 c.c. of C. P. 95 to 100 per cent methyl alcohol (acetone-free) for three to four hours. Filter and evaporate over water-bath to about 90 c.c. Titration of this antigen should be performed with strongly positive, weakly positive and negative serums, using dilutions of 1:2, 1:4, 1:8, etc., in 0.1 c.c. doses of each dilution. Nonspecific inhibition may occur in a 1:8 dilution or even higher but these extracts are powerful and not hemolytic. It may be cholesterolized if required with 0.2 per cent cholesterol. Because of precipitation, heat extracted antigens should be warmed prior to use. The antigen can then be diluted directly with physiologic saline. The dose employed is 0.1 c.c. of a dilution at least three times its inhibitory value. Titration of the antigen with a two-plus and three-plus serum is of great importance.

Patient's Serum.—The patient's serum is poured off from the clotted blood and centrifuged. The clear serum is pipetted off and inactivated at 55° C. for 20 to 30 minutes, the shorter period being less destructive of the "reagin."

Complement.—Pooled guinea pig serums are used, secured by bleeding from the heart or carotid as described by Karsner and Ecker.[8] For titration one c.c. of the serum is diluted with 29 c.c. saline using 0.03, 0.06, 0.09, 0.12, 0.15, 0.18, etc., in test tubes containing 1 c.c. or 0.5 c.c. of sensitized sheep corpuscles (to be

described later) and balanced with saline. The mixture is well shaken and incubated in the water-bath at 37° C. for one-half hour. The amount in the first clear tube, free from shadows and sediment, constitutes the unit. For use in reactions the remainder of the serum is diluted 1:10, and 2 units employed in the overnight fixation method.

Cell Suspension.—Wash 10 c.c. of whole defibrinated sheep blood four times with saline. Dilute the residual cells to 200 c.c. with saline or to 100 c.c. if only 0.5 c.c. sensitized cell suspension is to be employed. Standardize the suspension by determining the hemoglobin content with the Sahli method or by Hopkins tube as described by Ecker and Rees.[9]

Amboceptor.—The presence of natural antisheep hemolysin has been a matter of considerable difficulty in the hemolytic system because of its inconsistency (Kalisky,[10] Morgenroth and Sachs,[11] and Noguchi[12]). Noguchi for this reason chose an antihuman system. In an unpublished study Sasano and Ecker found that 19 per cent of serums contained two or more units of natural hemolysin and 19.6 per cent a noticeable amount but less than two units, while rarely serums containing four or more units are encountered. The Bauer technic[13] utilizing the natural amboceptor, has been discarded (Hoehne,[14] Meirowsky,[15] and Swift[16]). Cummer and Dexter[17] conclude that it is essential to take notice of presence or absence of this natural antibody in the performance of the test. Similarly Seelman[18] sees a source of error in the complement-fixation test in which an antisheep hemolytic system is employed. Simon[19] suggests the use of Noguchi's antihuman hemolytic system or preliminary extraction of the natural antibody. Kolmer and his collaborators, however, advocate the use of two units of amboceptor while Kahn[20] removes the natural hemolysin.

If the amount of amboceptor in the hemolytic system be large, not only is the required amount of complement reduced, but moderate increases in the quantity of amboceptor do not influence the reaction in any significant degree. This well established principle is a most important feature of the method here outlined. Those who have employed it (McKenzie,[21] Field,[22] Kaup,[23] Ranque and Senez[24]), have not sufficiently emphasized its value in the Wassermann test. Thiele and Embleton,[25] in a study of the mechanism of antibody action, have shown that increasing amboceptor diminishes to a certain degree the complement concentration necessary to produce lysis. Above a certain amount, increase in the quantity of amboceptor does not diminish the necessary complement concentration. It, however, greatly accelerates the rate of the reaction. Recently, Hill and Parker,[26] in agreement with Thiele and Embleton, pointed out that the main hemolytic reaction proceeds according to the law of unimolecular reactions and that the rate of reaction is, within certain practical limits, proportional to the square root of the amount of amboceptor per cell. The effects of increasing the amboceptor is, therefore, first to increase the "titer" of the complement, and second to increase the rate of the reaction. With increasing amboceptor the first effect reaches its limits earlier than the second. They showed that the complement concentration for complete hemolysis falls sharply with the first increments of amboceptor, but increases beyond about 4 or 5 M. H. D. produce no further effect in this respect. Hill, McKinstry and

Parker[27] in a recent modification of Harrison's method use 8 M. H. D. of amboceptor to sensitize their cells. Sasano and Ecker in a study of over 3,000 serums have found that with a well-balanced hemolytic system, an addition of as many as twenty units of amboceptor does not produce a complete hemolysis of one dose of sheep cell suspension, in a given time, when less than one minimum complement unit is present. Titrations of complement with cells sensitized with five units of amboceptor will completely eliminate the factor due to natural hemolysin, thus obviating the employment of an antihuman system or removal of the natural antibody. It was found further that in certain cases serums from which the natural antibody had been removed become more or less anticomplementary or weaker and some even negative. Again the use of five units of amboceptor does not in the least sacrifice the sensitiveness of the test and has the added advantage of a minimum quantity of complement. The amboceptor is prepared in the usual way, but bleedings are made five days following the last injection. The serum is inactivated at 55° C. for one-half hour and preserved with 50 per cent C. P. glycerol. It should be kept in an ice box, titrated often and have a titer of at least 1:1000. In titration, 0.5 c.c. of each dilution is added to 0.5 c.c. of the standardized cell suspension and 0.25 or 0.5 c.c. of pooled complement. In the reactions five units should be used.

Sensitization of Cell Suspension.—The necessary amount of amboceptor is added to 200 c.c. of saline and shaken. This solution is then added to 200 c.c. of the prepared erythrocytes and again shaken. If less than 1 c.c. (0.5 c.c.) of the sensitized cells are to be used, the cells may be suspended in 100 c.c. saline, the necessary amounts of amboceptor in 100 c.c. saline added, and the two mixed. The mixture is allowed to stand in a water-bath at 37° C. for thirty minutes prior to titration of complement. The writer uses 1 c.c. of first mixture.

METHOD

The technic is essentially the three tube system of Citron. The patient's serum is used in doses of 0.05 c.c. and 0.1 c.c., to which is added the antigen and complement. The control tube for patient's serum contains 0.1 c.c. serum plus complement and salt solution to balance. Two antigen control tubes contain respectively 0.1 c.c. and 0.2 c.c. of antigen dilution, together with complement and salt solution to balance. Controls should be set up with a known negative, a known two or three-plus positive and a four-plus positive serum, together with antigen and complement. The tubes are shaken and allowed to stand for five to fifteen minutes. Complement is then added in doses of two units and the tubes placed in an ice box at 4° to 8° C. overnight. The sensitized cell suspension is also kept in the ice box. The next morning the tubes and the cell suspension are warmed and the latter added to the tubes. The mixture is incubated for thirty minutes at 37° C. Readings are made at once and after sedimentation. The readings can be made with the aid of the Citron scale.

Spinal Fluid.—If the fluids be clear no inactivation is necessary. If they be bloody, they are centrifugalized and inactivated for fifteen minutes at 55°

C. If the quantity of fluid be sufficient, doses of 0.1 c.c., 0.25 c.c. and 0.5 c.c. are employed, and similar amounts are used in the controls. Experience has shown that with this method and overnight cold fixation, a maximum of 0.5 c.c. spinal fluid is adequate. Antigen dilution is employed in doses of 0.1 c.c. As with serum two units of complement are used. Saline is added to balance.

Quantitative Reaction.—This can be done by dilution of the patient's serum.

CONCLUSIONS

The method as described is not only highly specific, but has been shown by clinical correlation to be extremely sensitive, especially in relation to the treated cases. No false positive reactions have been recorded in examination of the serum of a large number of cases of scarlet fever and tuberculosis, as well as many normal pregnant women. Of particular interest is the case of malaria reported by McConnell[28] in which a positive reaction was obtained by this method, in spite of complete absence of clinical findings indicative of syphilis. At necropsy, however, there was an outspoken syphilitic aortitis with involvement of the aortic leaflets. As further refinements are made in the preparation of materials they can be adapted without essentially altering the basic principles of this method.

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A NOTE ON THE USE OF SOLID MEDIA FOR THE DETECTION OF GAS PRODUCTION BY BACTERIA. I*

By L. O. Dutton, M.S., and Velma M. Rutherford, A.B., Memphis, Tenn.

In a recent article, I[1] pointed out that there was a source of error in the detection of gas production by bacteria on agar-agar if the medium was allowed to dry to a certain extent On agar tubes that had been kept in the ice box for a period of fifteen days oı more, certain of the tubes failed to show gas bubbles when inoculated with a known fermenter of the sugar used. This result was thought to be due to the drying and resulting increase in the viscosity of the agar. It was suggested that to overcome this defect the media be stored in a humidor. An experiment was reported showing that media stored in such a container retained its ability to detect gas longer than the control medium stored in the ice box.

Having tried to overcome, practically, the too rapid drying of agar by storage in a very tight ice box in which the moisture content was maintained as high as possible, without success; and having met difficulties in the use of an efficient humidor large enough to be of service, some more simple manner to solve the problem was sought. It was thought that remelting the medium in boiling water and reslanting immediately before use might lower the viscosity to a point comparable with freshly made agar, and thus restore the efficiency of the medium for detecting gas production.

This was found to be the case, and upon trial, agar of an old batch revealed gas production readily. It remained only to prove that media stored in the ice box and then remelted, would give constant results in this regard. To arrive at the value of the method, a simple comparative experiment was done.

A number of tubes of dextrose agar were prepared with bromcresol-purple as the indicator of acid production. They were sterilized at twenty pounds pressure for twenty minutes. Immediately after slanting and cooling, ten of the tubes were planted with a culture of B. paratyphosus B. This was for the purpose of arriving at a more or less rough idea of the amount of gas to expect in the amount of medium employed, and to determine the limits of variability in this regard. The results were recorded in number of gas bubbles formed in the medium. Of course such a method is open to many objections but it is surprising how constant the number of bubbles will remain in a series of tubes, and how narrow will be the limits between which this number varies. The average number of bubbles produced in the ten tubes was fourteen and in each the medium was raised from the bottom of the tube. The largest number of bubbles recorded was seventeen and the smallest twelve. With this as a

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[1]Dutton, L. O.: A Note on Certain Limitations of the Use of Solid Media for the Detection of Gas Production by Bacteria. Jour. Lab and Clin. Med., September, 1924, ix.

basis, a series of cultures was made, inoculating two tubes, one of which had been freshly remelted and slanted on successive days. The results in regard to gas detection are shown in Table I.

It is clear that the remelted agar retained its ability to detect gas while the unmelted agar lost that quality almost completely. The striking difference between the two sets of media is not fully reflected by the number of gas bubbles alone. At no time during the twenty-eight days did the remelted agar fail to show a space between the bottom of the agar and the bottom of the tube. In contrast with this, the unmelted agar failed to show such a separation after the sixth day except for four days at irregular intervals. The small number of bubbles recorded in the older cultures on the unmelted agar were along the line of stab and in a study of unknowns would have been recorded as doubtful results. It is doubtful, in the light of past experience, whether the unmelted agar would have shown any bubbles at all, had the inoculations been made without stabbing.

The simple precaution of remelting and reslanting agar tubes that are to be used for the detection of gas production seems, then, to assure constant and accurate results.

In laboratories where large numbers of fermentation studies are done, there is probably a rapid enough turnover of the media stock to obviate the necessity of such a procedure. In those laboratories in which fermentation studies are done less often, however, there is a definite need for some method of keeping solid media that is to be used for gas detection in a fresh state. Errors in classification of species have come to our attention frequently, where the difficulty was traced to the failure of solid media to show gas. In our own laboratory several "new species" and "atypical variants" have turned out to be old friends, when care was taken in the fermentation studies.

Our conclusions are that there is ample opportunity for error in gas detection on agar by allowing it to be stored several days before use; and that in the interest of economy, both of time and materials, as well as the reliability of results, a procedure that will forestall such errors and at the same time allow the use of old media is desirable. Such a method is suggested, remelting just before use, and a simple experiment is detailed to prove that the method will prove efficient in practice.

TABLE I

TABLE SHOWING THE NUMBER OF GAS BUBBLES IN AGAR TUBES OF VARYING STORAGE PERIODS UP TO THIRTY DAYS. THE INOCULATIONS WERE MADE EACH DAY WITH B. PARATYPHOSUS B ON A REMELTED AND AN UNMELTED TUBE

Age of agar in days	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
No. of bubbles in remelted tube	8	19	15	14	12	8	12	7	16	8	9	17	13	17	16
No. of bubbles in unmelted tube	10	11	10	11	9	18	5	12	17	2	1	2	9	15	4

Age of agar in days	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
No. of bubbles in remelted tube	16	16	12	17	15	11	12	14	13	14	16	15	17	14	13
No. of bubbles in unmelted tube	13	3	3	3	1	4	1	3	2	2	1	1	1	1	1

AN ELECTRODE FOR MEASUREMENTS OF SKIN POTENTIAL*

By Walter C. Alvarez, M.D., B. L. Freedlander, M.D., and L. B. Clark, San Francisco, Calif.

IN 1882, Hermann[1] connected different parts of seedlings to a delicate galvanometer and found that the rapidly growing tips of the leaves and of the roots were negative (through the galvanometer) to the cotyledons. In recent years Child[2] and his associates have been studying similar gradients of potential along the axes of small organisms and have shown that they correspond to gradients of metabolic activity. Potential differences exist also between the various parts of the human body, but so far as we can find, no

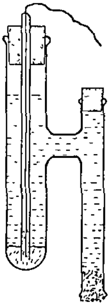

Fig 1.—Electrode described in the text.

attempt has ever been made to map them. It has long seemed to one of us that such a map might be worth making as it might throw light on the origins of the skin current. Relics of axial gradients might be looked for but we would hardly expect to find them in so complicated and so thoroughly integumented a structure as the human body.

As so often happens in research work, the little problem which was to take weeks took months and then ended in discouragement. Our only excuse for publishing this short note is that most of our labor went into securing a satisfactory electrode: one which we believe can now save other travelers along this road from much vexation and wastage of time. The difficulty which we found with all the various nonpolarizable electrodes, with zinc—zinc sulphate, mercury-calomel, ferric-ferrous sulphate, and other combinations, was that after a little contact with the skin they developed potential differences which made them too erratic for use. Success came finally through

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following a suggestion of Lund[3] who we found had just been through similar difficulties. We wish to thank him for his help so freely given. Our electrode which is depicted in Fig. 1 is a modification of his. In the bottom of the larger tube was placed a small amount of amalgam made up of 3 per cent lead and 97 per cent mercury. The smaller tube dipping into the lead contained a copper wire which was stuck into a small amount of stiff amalgam made of finely divided copper and mercury. The contact with the skin was made through a gauze wick which could easily be renewed together with the solution in the little side arm.

These electrodes remained equalized over long periods of time and when, occasionally, they did develop differences in potential we could quickly restore the balance by shorting them for a few minutes in a beaker of the

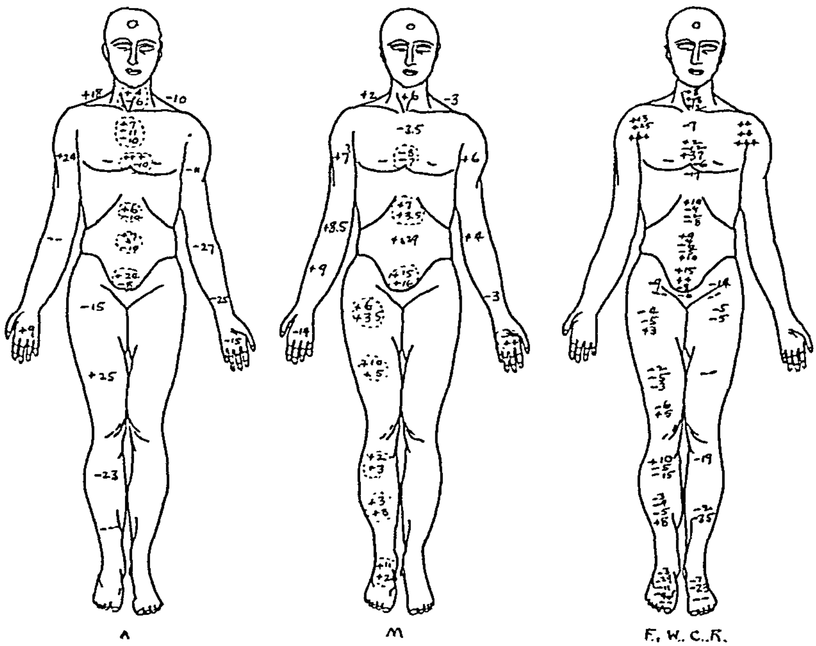

Fig. 2.—*A* and *M* show the data from two subjects. See text. The figures enclosed in circles represent measurements in the same place on different days. *F*, *W*, *C*, *R*, represent data from four subjects. The signs – – or + + indicate deflections beyond the galvanometer scale.

lead chloride solution. The readings were always made in duplicate with the positions of the electrodes reversed between times. When these readings were equal and of opposite direction we could be pretty sure of our results. A highly sensitive Leeds and Northrup d'Arsonval galvanometer was used.

Fig. 2 shows the type of chart which we have obtained. The readings are in arbitrary units of the galvanometer scale, and all are relative to the potential of a spot in the middle of the forehead. A double plus or double negative sign means that the deflection was too wide for the scale. When two or more figures are given in a circle they denote readings obtained on different days. The third chart in Fig. 2 shows data obtained from four subjects. These charts are not as complete as one might wish but even with

the electrodes working well it takes a good deal of time to be sure of a few measurements.

When it was found that these readings could be duplicated closely at intervals during any one session of a few hours it seemed as if they must be indicative of local peculiarities of some sort, but unfortunately they varied so markedly on subsequent days and in different subjects that we soon despaired of getting any definite information, and it seemed best to wait until more could be learned about the exact mode of production of the potential differences. A review of the literature shows that most of the work on this subject has been done by the experimental psychologists who have tried to find out why larger differences suddenly appear when the subject experiences a strong psychic stimulus. An excellent review of that work has been written by Prideaux.[4] Some of the more recent studies are by Gildemeister,[5] and Albrecht.[6]

Naturally, one of the first questions that arises is: how much of the current depends upon local differences in the intensity of perspiration? A glance at Leva's[7] charts shows that the sweat glands are most concentrated on the palms of the hands and the soles of the feet. There are large numbers also on the forehead and in the axilla. They are but few on the arms, thighs, legs and back. This distribution agrees with the findings of Waller[8] who, with the help of little watch crystals containing calcium chloride, measured the amount of perspiration in the different regions.

In our studies, we did find a good deal of correlation between the intensity of perspiration and the amount of current, but unfortunately, the regions of heaviest perspiration were sometimes positive and sometimes negative to the forehead, all of which is hard to understand. Furthermore, big potential differences could often be obtained between two places like the cheeks where there are few sweat glands. A careful washing of the skin made no change in the results. Warming the skin where one of the electrodes was to be applied had very little effect on the intensity or direction of the current.

SUMMARY

An electrode is described, suitable for studies on the skin current.

An attempt has been made to map the differences in potential in different parts of the surface of the body. The results so far are too erratic to give us much information as to the origin of these differences.

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A NEW USE FOR A VALENTIN KNIFE*

By William Snow Miller, M.D., Madison, Wis.

SOME two generations ago, before the advent of the microtome, the two-bladed knife of Professor Valentin was much used in obtaining thin sections for microscopic study. This was, in the hands of an expert, an efficient instrument for obtaining single sections.

The knife was made in two forms. The original form consisted of two blades, which were supposed to be capable of being so separated that the blades were parallel to each other; the distance between the blades being regulated by a screw (Fig. 1). When well made it was a serviceable instrument; otherwise a most unsatisfactory one. This form was later improved

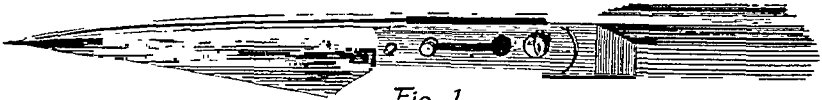

Fig. 1

Fig. 1.—The original of Valentin's knife. This example was made by a German cutler whose name has been obliterated through use.

Fig. 2

Fig. 2.—The improved form devised by Matthews. This particular knife was made by a Philadelphia cutler whose name was obscured when the knife was repolished.

by the addition of a second screw by means of which a more exact parallelism between the blades could be maintained.

An improved form was devised by an Englishman by the name of Matthews (Fig. 2). In this form the blades could be entirely separated from each other; a very desirable feature when it came to sharpening the blades and to cleaning them after use.

After more than a year's search I was able to resurrect an example of each type of the knife. Preference is given to the form illustrated in Fig. 2.

For several years I have been studying tuberculosis as it occurs in the human lung, and as it is produced experimentally in the lungs, and other organs, of the rabbit and guinea pig. I found it difficult to cut out blocks, with parallel sides, from a lung which contained very young tubercles, and not to include so much of the surrounding tissue as to make the cutting of a tubercle into serial sections and the mounting of them, a tedious task.

The idea came to me that a Valentin knife might be advantageously

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used for this purpose. I had used one in my student days, 1876-1879, but I did not own one.

I wrote to various dealers asking if they still kept in stock such a knife, and during vacation trips I visited instrument dealers in various parts of the country and asked the same question. In most instances I met with absolute ignorance of the existence of such a knife; by a few I was told they had discontinued making them. Finally I obtained from Professor Gage, of Cornell, the knife shown in Fig. 2, but in so dilapidated a condition that he feared it could not be made usable. However, it proved to be made of excellent steel and is now as good as new. Later I obtained from Pennock, of Philadelphia, the example shown in Fig. 1; but this proved to be so ill made that its use is limited.

I have found the knife illustrated by Fig. 2, exceedingly useful in cutting out blocks, with parallel sides, from one to five millimeters in thickness. If the edge be of the requisite keenness, it can be used on fresh lungs; but it works much better after the lung has been fixed and hardened, and this is the time when I make the most use of it. On the more solid viscera, as the liver or the kidney, it can be used advantageously any time.

In my hands the knife has proved so useful that it seems to me there is still a place for a Valentin knife in the histologic or pathologic laboratory, even though it be no longer useful in the work for which it was originally designed.

METHOD FOR CLARIFYING CLOUDY URINES FOR THE PHENOLSULPHONEPHTHALEIN TEST*

By E. A. Hewitt, D.V.M., St. Paul, Minn.

THE clinical determination of renal function by means of phenolsulphonephthalein was devised by Rowntree and Geraghty and depends upon the injection into the tissues of a dyestuff which is eliminated rather rapidly by the normal kidney, and can be estimated quantitatively in the urine.

The technic usually employed is as follows:

1. Give the patient 300 to 400 c.c. (about two glasses) of water to promote secretion of urine.

2. Twenty minutes afterward have him empty his bladder and discard the urine. Then inject intramuscularly 1 c.c. of sterile phenolsulphonephthalein solution. This may be secured in ampules from Hynson, Wescott and Dunning.

3. In exactly one hour and ten minutes from the time of the injection have the patient empty his bladder and save all the urine. The ten minute

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period represents the usual time which elapses between the injection and the first appearance of the dye in the urine.

4. In two hours and ten minutes after the injection, the bladder is again emptied and the urine saved in a separate container.

5. To estimate the output of phenolsulphonephthalein, the volume of each of the one hour portions of urine is recorded; to each of the two portions is added sufficient 10 per cent sodium hydroxide solution to bring out the maximum purplish red color, and each portion is made up to a volume of 1000 c.c.

A standard is prepared by adding 1 c.c. of the phenolsulphonephthalein solution to about 800 c.c. of water; this is alkalinized by sodium hydroxide and diluted to 1000 c.c. The unknown is read against the known in a colorimeter. Usually, percentage solutions of the known are made up and compared against the unknown.

An adaptation of this method was applied to cattle but it was found that when the urine was treated with sodium hydroxide, and diluted to 1000 c.c. in each case it was so cloudy that no colorimetric reading could be made. This cloudiness could not be removed by any filtering process that would not take out the dye.

It was the belief that this cloudiness was due to the suspended sulphates and phosphates in the urine, which are contained in abundance in ox urine.

After trying several methods of precipitating these sulphates and phosphates without success, it was suggested that barium hydroxide be tried, as these constituents are precipitated by barium hydroxide. Accordingly one part by volume of a saturated solution (5 per cent) of barium hydroxide, was added to five parts of urine, after which the material was filtered with the result that the filtrate maintained the original color and was a clear transparent liquid.

A standard solution was then made up using 100 c.c. of a saturated barium hydroxide solution, dissolving one c.c. of the phenolsulphonephthalein solution and the volume made up to 1000 c.c.

It is suggested that this method might have an application in the routine procedure of the phenolsulphonephthalein renal function test in its application to humans.

A NEW METHOD FOR STAINING ELASTIC FIBERS*

By Joseph M. Thüringer, M.D., Norman, Okla.

AMONG the various stains for the demonstration of elastic tissue the Resorcine-Fuchsin method of Weigert[1] and the Orcein method of Unna-Tänzer with their numerous modifications are familiar to every worker. The preparation of the Resorcine-Fuchsin stain is a somewhat tedious process requiring much time though the staining rarely takes more than an hour.

The Orcein method of Tänzer[2] or Unna[3] requires staining from six to twenty-four hours at room temperature although it may be hastened by staining in a moderately warm incubator or over a flame until the stain thickens.

Harris[4] makes use of "Elasthaematin" which is a stain composed of hematoxylin and aluminum chloride, ripened with mercuric oxide and acidulated with HCl.

The above mentioned and numerous other methods while yielding good results are open to many objections the principal ones of which are:

1. That a special stain must be kept on hand.
2. The loss of time in the preparation of the stain.
3. The length of time required for staining.
4. Some of the methods are not suitable for the simultaneous preparation of a large number of sections or slides.
5. Since the stain is not frequently employed it is usually unfit for use when desired.

To overcome these objections I have looked for a method which would permit that the ordinary routine hematoxylin-eosin stain be adopted for this special purpose.

The sections are given a preliminary treatment as follows:

(a) Place in one-half of 1 per cent phosphotungstic acid for five minutes.

(b) Rinse well in distilled water.

(c) Stain deeply in Mann's hematin three to five minutes.

(d) Differentiate in 2½ per cent acetic acid in water.

(e) Rinse in distilled water.

(f) Stain with eosin.

(g) Dehydrate and clear.

The entire procedure varies from a routine hematoxylin-eosin stain only in the preliminary treatment of the sections in one-half of 1 per cent phosphotungstic acid solution in water.

Mann's hematin is the formula employed in my laboratory for routine work. It is essentially similar to Ehrlich's acid hematoxylin but is made up

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from hematin instead of hematoxylin. It is ready for instant use and five-year-old solutions on hand show no signs of deterioration.

Formula for Mann's acid hematin

Hematin	1.25 gm.
Glacial acetic acid	10.00 c.c.
Glycerine	100.00 c.c.
Absolute alcohol	100.00 c.c.
Potassium-alum sulphate	10.00 gm.
Water	100.00 c.c.

Dissolve the powdered hematin in the acetic acid; if necessary add 50 c.c. of absolute alcohol. Then add the glycerine, rinsing the measuring cylinder with the remaining 50 c.c. of the absolute alcohol, which also add.

Dissolve the alum in the water with the aid of heat and when partly cooled mix with the above and filter. The stain is now ready for use.

The elastic fibers are stained deep blue and appear in great contrast to the general pink of the remainder of the section. The nuclei stain red but are unobtrusive. The chromatin granules vary from light brown to blue.

The elastic lamina of arteries show beautifully as well as the most delicate elastic fibers scattered throughout the tissue.

Delafield's hematoxylin may be used if it is desired to show nuclei with greater contrast.

Hansen's hematoxylin is especially recommended if a brilliant nuclear stain is desired at the same time. The contrast between the elastic fibers and other tissue elements, however, is greatly diminished.

Phosphomolybdic acid must not be used in place of phosphotungstic acid since its "affinities" lie more in the direction of the collagen fibers which stain very deeply with the hematoxylin after preliminary treatment with dilute solutions.

SUMMARY

1. The above method gives a very specific stain for elastic tissue.
2. Any section which takes a good hematoxylin-eosin stain will yield good results.
3. Any of the common fixatives may be used, Zenker's, Helley's, formalin, alcohol, etc.
4. No special stain has to be prepared. It is only necessary to "mordant" sections for five minutes in one-half of 1 per cent phosphotungstic acid in water before staining with hematoxylin.
5. This method gives uniformly good results with paraffin, celloidin or frozen sections.

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EDITORIALS

Heart Block

THAT there is romance in the history of medicine no one may deny although the story may not have the content or even the form of the everyday one of love and adventure. But the romances of medicine are not as popular, are not as well known, are not, perhaps, as easily understood as the "best sellers" of current fiction. If this were not so the works of Brown-Séquard would be as well known as the Henty books or the Nick Carter series. Perhaps Gertrude Atherton will popularize certain aspects of medical research from the beginning with *Black Oxen*. It is a pity that Franz' *Through the Ivory Gate* is not read more. It is to be regretted that Freud is looked upon as erotic rather than as romantic.

The romances of medicine appeal perhaps only to the active workers in science. Sometimes when they are read by the student they are the immediate cause of a new career in science. Often they speed up the enthusiasm of the middle-aged routine worker or bring new thrills to the tired researcher.

Only occasionally someone writes an essay or even sometimes a book that is positively thrilling. Such essays are certain ones of Osler, of Daniel Drake, of Darwin, of Huxley. Such books are exemplified in Vallery-Radot's *Life of Pasteur* and in Cushing's *Life of Osler*. The whole career of Paracelsus is romantic even when one subtracts the halo Browning built about it. It was romantic to Edith Wharton whose poem on Paracelsus is a jewel. The more popular medical stories belong to the field of the practice of medicine. Few of them relate to the laboratory aspects of scientific labor. It seems popularly true that the sentimental relations of physician and patient should be the central theme as exemplified in *Rab and His Friends* and very many other stories. Perhaps *Black Oxen* is evidence of change in a new direction, and *Dracula* in another. At any rate even in addresses for physicians themselves it is usual to call attention to the results that they have had with the products of laboratory research, which is to say, the end-result is apostrophized rather than the trials of production. Hence it is perhaps that Lister is better known even to physicians than Pasteur or Liebig, and that few physicians know Theobald Smith, the pioneer in the study of transmission of disease by insects. When both aspects, the experimental (the so-called theoretical and underpaid) and the therapeutic (the so-called practical and overpaid) are used together, a complete scientific romance may be the result as in the gorgeously true *Arrowsmith*, a story of the "main street" that runs about the Olympus of medicine. The ordinary accounts of the trials, tribulations and successes of medicine are like the recent address of Haggard oratorical patchworks elaborated from bits of biography used for the purpose of professional sentimental exaltation. They belong with the Fourth of July orations.

In days gone by scientific progress was largely an individual affair, each step in advance the result of the efforts of a single worker testing his vision of what might be. Now it is become a matter of teamwork, of cooperation in research. Modern science depends for progress upon this teamwork which is the new factor added to the old one of intellectual liberty. Sabin has made a stimulating plea for this combination.

One of the most recent romantic fragments and one which shows the value of cooperative research, is to be found in a yearbook! In his report for the Department of Embryology of the Carnegie Institution of Washington, Dr. Streeter reports certain researches on the physiology of the heart. Let me quote: "It was found by Mr. Johnstone that delicate ligatures could be placed at different levels around the beating hearts of chick embryos and that he could thus functionally isolate the different parts of the heart tube, producing what is known in the adult as *heart block*, a condition where the subdivisions of the heart beat independently of each other, the coordination normally existing between ventricle and atrium being lost. Since this could be done in embryos of 2, 3, and 4 days' incubation, it became possible for Mr. Johnstone to study contraction of embryonic heart-muscle before the problem is complicated by the ingrowth of nerve fibers, and where the only conducting system is the muscle itself. Further than that, by altering the level of the ligatures, the different parts of the heart tube could be

observed, both separate and connected, and thus the effect of one part on another could be determined for different stages of growth.

"Mr. Johnstone found that when a ligature is tied at the atrio-ventricular junction, the atrium continues to pulsate, while the ventricle and bulbus, after stopping for a moment, begin again to contract, but at a slower rate and more irregularly than that portion of the heart on the other side of the ligature—that is, a definite heart block is produced. If, after the ventriculo-bulbar segment has resumed its contracting, another ligature is placed around the heart at the junction of the ventricle and the bulbus arteriosus, the latter stops contracting while the ventricle continues without apparent change. The bulbus in these experiments sometimes fails to beat again, but if pulsation is reestablished it is after a longer interval and at a slower rate than in the isolated ventricle.

"At present there is some uncertainty as to the exact location of the junction of the sinus venosus with the atrium in these early stages, and this is one of the problems that now faces us. Mr. Johnstone placed a ligature in this general region and all that part of the heart tube cranial to the ligature (atrium, ventricle and bulbus), after a brief cessation, resumed pulsations, maintaining the regular sequence of atrium, ventricle, and bulbus, though at a slower rate than before tying the ligature. It would thus appear that the ultimate and regulative control of the heart beat must be in the sinus region.

"Further experiments were done in which the ligatures, after being firmly tied, were removed, leaving a crushed line of constriction in the cardiac tube that physiologically isolated the portions on either side of the constriction, producing heart block in accordance with the level at which the crushing occurred.

"Dr. Lewis has studied the initiation and character of the early rhythmic contractions of the heart by removing this organ from 2- and 3-day chick embryos and observing its behavior in Locke's solution, where it maintains fairly regular automatic pulsations for several hours at room temperature (26° C.), varying in different hearts from 40 to 80 per minute, which is about one-third of that in the freshly opened egg. If the temperature is gradually increased the rate increases, going from 26° to 30°, about 4 beats per minute, from 30° to 35° from 4 to 8 beats per minute, and from 35° to 40° from 4 to 20 beats per minute. The rate of the isolated heart varies definitely according to certain environmental conditions, such as temperature, carbon-dioxide tension, hydrogen-ion concentration, and salt content of medium, and also upon mechanical stimulation.

"In studying the effect of changes of temperature on contraction phenomena of these isolated hearts, Dr. Lewis found that the heart block which Mr. Johnstone had effected by ligatures could be brought about simply by an abrupt rise in temperature. For example, when a heart beating regularly at 92 per minute at 27° C. was placed in a warm box at 38° C., after three minutes the atrium was beating at 120 and the ventriculo-bulbar part at 12 per minute; and when returned to room temperature, within ten minutes the whole heart was found beating synchronously again at 92 per minute. There

was some variation in the behavior of the individual specimens as to the degrees of temperature necessary and the amount and character of the disharmony, but in general the effect was as striking as the illustration given. With few exceptions the atrial end was most resistant and beat most rapidly and the ventricular and bulbar portions, in that order, exhibited a much slower rhythm and showed more tendency to be intermittent. The heart block of the clinicians has been supposed to be due to injury to the atrioventricular conduction bundle. Since these young embryonic hearts contain no such bundle, it cannot be the explanation here. Nor is it due to any serious injury of the atrio-ventricular or ventriculo-bulbar junctions, since the condition disappears when the hearts are brought back to room temperature. The explanation must rest in the physiologic differences existing between the muscle cells of the atrium, the ventricle and the bulbus. Apparently these physiologic differences are increased through the circumstances of the experiment and the different parts of the heart are rendered more sensitive to thermal and other changes. It is certainly not temperature alone that causes heart block, because the same temperature in the egg, with the heart in the normal environment, yields a synchronously beating heart.

"The inherent difference in the constituent cells of the atrial, ventricular and bulbar portions of the cardiac muscle-sheet is best shown by dividing the isolated heart into these three parts and studying them in Locke's solution. Dr. Lewis made records of such preparations, lying side by side, under various conditions of temperature and in open and closed chambers. This latter point makes a difference, for when the observing chamber is closed with a cover-glass the rate is accelerated, due presumably to increased carbon dioxide tension. It is hoped that this important point can be definitely determined. When kept at room temperature it was found, in confirmation of Mr. Johnstone's ligature experiments, that the atrium always beats faster than the ventricle and the ventricle faster than the bulbus. The rate of the atrium approaches closely that of the entire heart. The rate of the ventricle, on the other hand, is usually less than half that of the atrium for the 2-day hearts, and considerably less than that for the 3-day hearts. The ventricular rhythms are apt to be more irregular than the atrial. The pulsations of the bulbus often fail entirely and are usually few in number and irregular when they do occur. All of these things make it quite evident that the relatively fast rate of the ventricle and bulbus in the intact heart is dependent upon stimulus from the atrium. When these parts are separated they show less spontaneity in older stages than younger ones. In other words, the muscle cells of the ventricle and bulbus tend to lose their spontaneous rhythmicity with age."

Isn't that romance?

—*P. G. W.*

Cancer and the Filterable Virus

WITH the arrival of the transactions of the ninety-third annual meeting of the British Medical Association we find instructive details on the work of Gye and Barnard which has recently been heralded in the lay press. Dr. William Ewart Gye of the Department of Experimental Pathology of the National Institute for Medical Research at Hampstead, presented the results of his studies before the section on pathology and bacteriology.

Years ago, contagious disease was thought to depend for its origin merely on the juxtaposition of host and parasite. While this still holds for many infections, exceptions do exist. Tetanus bacilli washed free from toxin are harmless. But if sufficient toxin is introduced together with the germ, tetanus ensues. The same is true of B. welchii infections. Yet a third factor sometimes plays a part, the factor of the body tissues or tissue resistance. Fifty per cent of a population may be carriers of the meningococcus but remain free from meningitis. The same occurs in amebiasis, diphtheria, typhoid fever and other diseases.

Cancer has been held to be a specific disease entity in part because a tumor arising in one animal can only be transmitted to another animal of the same species and only after the injection of living cancer cells. On transplantation the characteristics of the parent tumor are faithfully reproduced. Opposition to the infection hypothesis has been made chiefly by those students of cancer research who have been interested rather in morphologic studies and whose chief concern has been the specificity of the different types of malignant disease. All this has been considered evidence against the germ theory of cancer, but Gye points out one important exception among animal and experimental cancers. This is the chicken sarcoma discovered by Peyton Rous in 1911 which was transmitted by the implantation of dead sarcoma cells or even by the injection of cell-free filtrates of the sarcoma. Two different chicken tumors have since been discovered by Rous, in each of which the sarcoma produced by filtrates always retained the same characteristics as the original. Two are spindle-cell sarcomas and the third an osteochondrosarcoma.

Gye has discussed at considerable length the difficulties and drawbacks associated with the study of so-called filterable viruses. Much has been done with these forms of life since Loeffler and Frosch discovered, in 1898, the first filterable virus in foot and mouth disease, but many of our concepts still appear erroneous. By many filterability is still considered the final criterion. There are, however, other factors which are even more important. Thus in herpetic encephalitis the saline filtrate from the herpetic vessicle is active, while the brain tissue thoroughly ground with sand and diluted with saline is noninfective after filtration. He suggests that here the virus has become so firmly attached to the brain tissue that it cannot be separated by ordinary mechanical processes. McCartney disagrees on this point, ascribing the noninfectivity of brain tissue filtrate rather to the presence of lipoid material in the macerated solution which clogs the pores of the filter. He points out that after preliminary incubation in glucose

broth, the virus passes from the tissue into the surrounding medium and will then pass through the pores of a Berkefeld filter.

Gye points out that in vaccinia, which is undoubtedly a virus infection, the filtrate from calf lymph does not contain the virus. He suggests that perhaps the virus becomes attached to the contaminating cocci or that it has the property of being adsorbed onto the surface of the filter. Gordon, who has done much work on the virus of vaccinia, in discussing this phase of Gye's communication, states that if *sufficient* filtrate be used the presence of virus in small quantities can be proved. If the suspension of virus be autolyzed before filtration, the virus will come through. It may also be liberated from the cells by preliminary trypsinization. He also records that the virus is readily adsorbed by the filter itself and concludes that there can be no reasonable doubt that vaccinia is due to a filterable virus. Coplans points out the marked absorptive properties of recently baked filters.

But, returning to the work of Gye, he has had no great difficulty in filtering what appears to be the virus of chicken sarcoma. This is confirmatory of Rous' work. Success in filtration requires a sufficiently great dilution and the use of salt solution rather than distilled water. Hydrogen-ion concentration appears also to be a factor governing filterability. Acidity hinders filtration while alkalinity makes the process easier. Apparently the properties of the liquid in which the virus is suspended play an important part.

The specificity of tumor cells is remarkable. But just as remarkable is the fact that cell-free filtrates of chicken sarcoma are equally specific. They produce tumor in no other bird than the hen and best in that particular variety from which they were first isolated, the Plymouth Rock.

The author suggests that in cancer as in tetanus there may be two essential factors, the filterable virus and the tumor tissue itself or at least some derivative of the tumor cells. He brings forth evidence that a chemical accessory factor is necessary for the production of tumors. The Rous sarcoma tissue becomes noninfective in primary broth culture within from two to seven days. Gye felt that this might be due not to the death of the virus but to the disappearance of an accessory chemical factor. Rous had observed that chloroform and certain antiseptics destroy the infectivity of filtrates, presumably by killing the virus. Gye, therefore, treated a fresh filtrate with chloroform to destroy the virus. Theoretically, the *chemical factor* was still present in the filtrate. This chloroform treated filtrate when injected into a hen caused no tumor. A noninfective primary culture presumably containing *the virus* also caused no growth. The two injected together in equal parts did result in tumor growth.

After centrifuging a filtrate at 9,000 revolutions per minute for two hours, the sediment was found to be infective while the upper layers were not. This suggests strongly the particulate character of the virus. If now the sediment is washed with saline and repeated centrifuging the sediment which still contains the virus but none of the chemical factors in the solution, is no longer infective. Neither is the supernatant fluid from the original centrifugalization which presumably contains chemical factor but no virus. Both together, however cause tumor growth. One of the factors is thrown

down in the centrifuge while the other is not. This suggests that the latter is indeed a chemical substance. Neither operating alone will cause sarcoma.

Gye believes that since the virus alone is not infective, the specificity of the Rous tumor must depend upon a chemical factor derived from the tumor cells. That it is actually derived from the tumor cells is suggested by the observation that subcultures are not infective. It is only the original or primary culture which contains tumor tissue that continues infective when used alone. Gye believes that the virus in the three forms of Rous chicken sarcoma is probably the same in each case, while the chemical factors are different. The factor is a cell derivative which may be separated from the cells.

He appears to have demonstrated satisfactorily that the particulate factor, the virus, will multiply in vitro. With the aid of KCl broth and chicken embryo subcultures have been made from the primary culture. A fifth subculture will have diluted the original inoculum one thousand billion times. While an eighth subculture causes no tumor, and a chloroform-treated filtrate of a primary culture (containing the chemical factor alone) causes no tumor, this treated filtrate *plus* the eighth subculture caused the usual tumor in the customary length of time.

The work on mammalian tumors has been unusually interesting. A spindle-cell mouse sarcoma, a rat sarcoma, a mouse carcinoma and a rat carcinoma were studied.

While the filtrate from mouse sarcoma will not cause sarcoma in the mouse, a recent unfiltered primary culture thereof will produce a growth. However, if the primary culture be maintained under *strict* anaerobic conditions, the filtrate *will* cause tumor growth. Thus mammalian sarcomas can likewise be transmitted with a cell-free filtrate and they thereby resemble the Rous chicken sarcoma. The difference lies in that the accessory chemical factor is either more abundant or more stabile in the chicken sarcoma. The evidence points to a destruction of the chemical factor by oxidation and it may be that strict anaerobic conditions preserve the chemical factor in greater quantities.

Gye has thus shown that mammalian sarcomas behave like chicken sarcomas provided the chemical factor is present in sufficient quantity and is not destroyed by manipulation.

In his work on the rat sarcoma and the rat and mouse carcinomata, the author employed the chemical factor obtained from the Rous chicken sarcoma. He did this primarily because the chemical factor appears to be more abundant in the chicken sarcoma. The cultures used were from the mammalian tumors and theoretically contained the virus and little or no specific chemical factor. The injection into chickens of *mammalian* virus with the chicken sarcoma chemical factor causes a tumor growth that can in no way be distinguished from the original Rous chicken sarcoma. Gye concludes that the virus obtained from each of these five sources reacted alike and that the chemical factor determined the specificity of the growth. He does not give protocols describing the injection of mammalian virus and Rous chemical factor into mammals. This should serve as a control for the injections into birds.

Three human tumors have been studied. The culture of the first from a scirrhous carcinoma of the breast was contaminated. The second, from a sarcoma of the thigh gave negative results. Cultures were made from the third, an adenocarcinoma of the breast, and after injection into a chick of this culture, together with the Rous chemical factor, a tumor resulted which was indistinguishable from the Rous tumor. As a control the chemical factor alone did not cause tumor.

It should be noted that cultures rather than filtrates of the mammalian tumors were used in the inoculation experiments.

Gye contrasts his specific factor with the commonly known aggressions. The toxin of B. welchii may be taken as example of the latter. Bacilli washed free from this toxin are harmless to mice. Washed cultures of B. welchii to which has been added a sublethal dose of the toxin, produce a characteristic spreading gangrene with toxemia. The disease is characteristic of B. welchii infection. This same toxin added to vibrion septique causes the disease characteristic of vibrion septique infection. Toxin-free harmless tetanus spores mixed with sublethal doses of B. welchii toxin caused tetanus. The specificity of these diseases depends upon the germ while the chemical agent is nonspecific. This would appear the reverse of the situation with Gye's cancer virus.

He accepts in part the suggestion made by various workers that the filterable viruses act upon cells in a different manner from the larger microbes. They are probably intracellular during their existence in the host in contrast to the bacterial microorganisms which exist chiefly in the connective tissues.

He feels that the virus of chicken sarcoma is corpuscular in nature, particularly since after prolonged centrifugalization the lowermost layers of the fluid cause more rapid and extensive growth than the uppermost. (The rapidity of appearance and growth of this tumor depends on the amount of virus implanted.)

He finds that the organism cannot be stained and examined with the ordinary microscope or with the routine staining methods such as Giemsa's or polychrome methylene-blue. The microscope is of value only in revealing the presence of contaminating germs. Barnard, however, has constructed an ultramicroscope employing ultraviolet rays, with which he has clearly photographed objects less than 0.2 micron diameter. He describes the virus as a small spheroid, which appears to reproduce by a process somewhat akin to "budding." Small particles develop at the periphery of the spheroid and gradually become loosened after remaining for a time attached by a very fine filament. The particles appear later to become new spheroids. Photographs show a magnification of 2200. The author estimates the diameter of the particles as less than 0.075 micron. The spheroids have roughly five times this diameter.

In view of recent statements intimating that Barnard was a merchant who had happened upon his discovery somewhat by accident, it should be noted that he has made various contributions in the medical literature, that

he is associated with the National Institute for Medical Research and is a Fellow of the Royal Society.

Gye believes that his virus is not a phase in the life history of a larger organism although his observations so far naturally cannot be conclusive on this point.

Several pertinent points were raised by Dr. J. A. Arkwright in his discussion of Dr. Gye's paper. Dr. Arkwright said: "As a general comment on the discussion it seems to me that there should be no assumption that the viruses known as "filter-passers" are all of a similar nature. In regard to many of them it may be said that we have little reason to regard them as similar except our ignorance of their nature, including our inability to see them. Next, I should like to express my unbounded admiration for the work of Dr. Gye and Mr. Barnard on the cause of cancer. This does not mean that I think there will be no criticism of their interpretation of the experiments they have published. Whilst their facts are no doubt correct the hypothesis will, I feel sure, be subjected to critical examination. There are two points on which I should like to ask Dr. Gye to give us further enlightenment. The first question, which it seems almost certain he will answer in the affirmative, is whether the experiments, in which Rous' fowl sarcoma was produced by the inoculation of a chloroform-treated 'sand filtrate' of one of these tumours together with a 'subculture' of the Rous 'virus,' were controlled by giving parallel injections of material in which uninoculated culture medium containing fresh chicken embryo was substituted for the 'subculture.' Another point which strikes one is the resemblance between the chloroformed-sarcoma extract, which Dr. Gye does not regard as living and calls the 'specific chemical factor,' and some of the so-called 'filter-passing viruses' which are associated with various other diseases. Some of these are remarkably resistant to adverse conditions. For example, the virus of vaccinia is well known to have a high resistance to chloroform and ether, and the virus of foot-and-mouth disease is known to resist drying over sulphuric acid, and was found by Loeffler sometimes to withstand 1 per cent carbolic for many weeks. If the 'specific factor' is really a simple chemical substance, as Dr. Gye believes, it is remarkable that it should be able to determine the kind of cell in the new growth resulting from an inoculation and the kind of animal in which the virus can cause growth."

Dr. Gye said: "The answer to Dr. Arkwright's first question is 'Yes.' Parallel experiments have been done many times with negative results. The second question is more difficult. The interpretation put upon the experiments has been founded upon: first, cultures from chloroformed extract fail; secondly, direct dilution experiments are all favourable to the interpretation given; and thirdly, the assumption that the effective factor is another virus again leads us to the position that strictly specificity is dependent upon a virus. In short, the simplest and most direct interpretation has been put forward, and this is supported by available facts."

The work of Gye and Barnard is stimulating. Their experiments must of course be confirmed. If such confirmation is forthcoming it matters little whether the explanatory hypothesis will require modification. The essential

point is that, while the morphologic pathologists have to a certain extent reached an impasse and the outlook for early progress has appeared doubtful, the resurrection of the infection hypothesis opens new avenues of approach and study.

Work on filterable viruses is a most delicate procedure and being dependent on a large number of factors, requires a greater variety of checks and counter checks than the authors have as yet applied.

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—*W. T. V.*

Rural Hospitals

With the purpose of improving rural medical nursing and hospital care, the Commonwealth Fund of New York is offering to assist in the building of one hospital in a rural section. Certain conditions are laid down governing the distance from existing hospitals, number of physicians for staffing the hospital, character of highways and transportation lines, willingness and ability of the community to defray deficits from operation. The Fund offers to pay two-thirds of the cost of construction and equipment.

If the experience in building one such hospital seems to warrant an extension of the program, the Fund will consider making a like offer to other districts needing a hospital. Applications are now being received, and considered. It is probable that a decision as to the first unit will be made in the early fall.

Erratum

On page 496, March, 1925, issue, Dr. Wadsworth's article, the third line in the tabular matter at the bottom of the page should read:—1 c.c. 4N HCl = 40.00 c.c. N/10 HCl

The American Society of Clinical Pathologists

Dallas Chosen for Next Convention

Responding to the cordial invitation extended by our colleagues in Texas, the Executive Committee of the American Society of Clinical Pathologists and the newly elected officers have unanimously chosen Dallas as our meeting place for the 1926 convention. The date has not as yet been definitely settled but it will permit our members to attend the annual American Medical Association meeting which will be held in the same city.

The selection of Dallas for our next convention is a well merited tribute to the Texas contingent of the American Society of Clinical Pathologists who were among the most active in the organization of our society and the first to form a state association of pathologists, thus stimulating the formation of our national organization.

Socialization of Clinical Pathology

One of the topics under consideration at the business session of the last Convention of American Society of Clinical Pathologists which elicited considerable and spirited discussion was the encroachment of state and municipal laboratories in the domain of the clinical pathologist. The socialization of medicine has been going on apace. One hears protests from the profession on the invasion of State Medicine in the field of general practice. The complaint is not limited to the medical profession. In industrial, manufacturing, and commercial spheres we hear a good deal of state interference with private initiative.

It is not within our province to enter here into political economy and dilate on the relative merits of state versus individual operation. The business people can take care of themselves. The medical profession at large i

making itself felt through the American Medical Association against the competition of state medicine.

Clinical pathology will have to fight for its rights in the competitive struggle. The principle here involved is not necessarily the vulgar bread and butter question but the interest of the patient. Socialization of clinical pathology with the concomitant low compensation will not attract the best talent, who will seek other fields. The result will be an incompetent personnel with consequent injury to the welfare of the patient.

Here is where organization can be effective in combating socialization of clinical pathology. The appointment of a Committee by the American Society of Clinical Pathologists to study the problem is a step in the right direction.

Society Notes

The Society maintains a Service Bureau for its members. Any clinical pathologist wishing to make a change should communicate with the Secretary.

Kindly report any change of address to the Secretary.

The next annual Convention of the American Society of Clinical Pathologists will be held in Dallas, Texas, April 15, 16, 17, 1926.

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CLINICAL AND EXPERIMENTAL

VALUE OF THE LEUCOCYTE FRAGILITY TEST IN THE PROGNOSIS OF PNEUMONIA*

By C. A. Pons, M.D., and E. P. Ward, M.D., Philadelphia

CONSIDERABLE attention is given by the clinician to the total leucocytic count and to the estimation of the percentage of the various cells in the blood as an aid in the diagnosis, treatment, and prognosis in certain conditions. While exceptions to expected figures are not infrequent, a high leucocyte count when accompanied by a proportionate increase in the polymorphonuclears is interpreted as an indication of high resistance. Conversely such conditions which *should* be accompanied by a high leucocytic count, when they fail to be so accompanied tend to end fatally.

If the number of leucocytes alone is taken as the index of resistance to infection, the inferences drawn must necessarily often be faulty; and any test that would tell us something *more* about the efficiency of those cells which aid in combating infection would naturally be a valuable addition. Indeed there have been many such efforts; namely, Walker's index of resistance, Wright's opsonic index, the Arneth formula, and its modifications, all aiming to secure more data from hematologic studies.

Another perhaps more valuable method of study is the determination of the so-called fragility of the leucocytes.

In 1916, Mauriac[1] described a technic for the determination of the fragility of the leucocytes, later with Carbonet and Moreau[2] two other papers were published. They proposed an index of fragility and its behavior under various conditions and diseases studied.

Mauriac was particularly interested in a phenomenon which he called

*From the Laboratory of Clinical Pathology of the Philadelphia General Hospital.
Read before the Fourth Annual Convention of the American Society of Clinical Pathologists in Philadelphia, May 20-23, 1925.

"Oscillation of Defense." He observed a fluctuation in the curve of fragile cells coincident with certain critical stages, such as the crisis of pneumonia, the clearing of the spinal fluid in the purulent meningitis, etc. This oscillation is characterized by a diminution in the number of the fragile cells prior

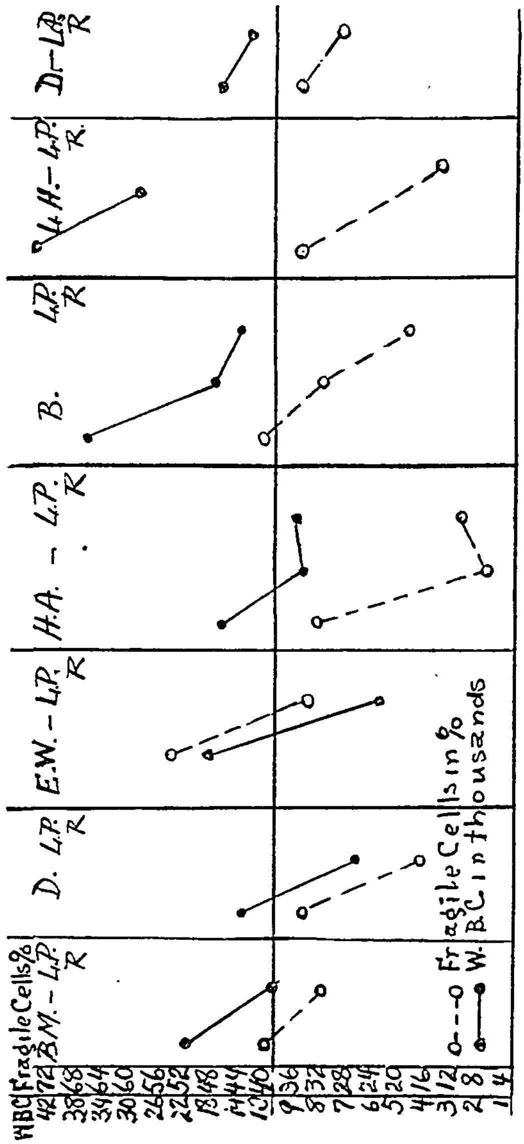


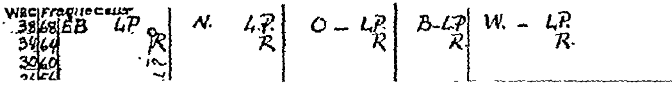
Fig. 1.

to crisis, followed by a sharp increase at the time of crisis and a postcritical fall to about their former level.

Mauriac associated this increase in the fragile cells with a rapid liberation of ferments and antibodies. While we have occasionally observed this so-called *oscillation of defense,* we are not prepared to confirm or disprove Mauriac's theory. However, we think that this increase of fragile cells may be the result of lessened demands for the delivery of new cells into the blood stream, as the disease attains its evolution and the patient recovers; in which

case the rapid rise in fragile cells would be the *effect* of the crisis and not its cause.

Recently Sampson,[3] of the University of California, has published a comprehensive study of the subject and modified the technic so that it is scarcely more complex than an ordinary leucocyte count.



EXPLANATION OF ILLUSTRATIONS

Fig. 1.—Group 1. Pneumonia patients who recovered The fragile cells are at or below the danger zone; as the disease progresses to a favorable termination the number of fragile cells decreases. The leucocytic curve parallels the fall in the fragile cells.

Fig. 2.—Group 2. Pneumonia patients who recovered. The fragile cells are below the danger zone and rise to or about normal This series shows that the curve of fragile cells is not dependent on the leucocytic curve.

Fig. 3.—Group 3. Pneumonia patients who recovered The fragile cells are below the danger zone. There is a rise in the number of fragile cells at crisis, and a postcritical fall This is Mauriac's Oscillation of Defense.

Fig. 4.—Group 4. Pneumonia with complications This group shows a rise in the *fragile cells prior to or with the onset of complications*

Fig. 5.—Group 5. The [illegible] *the fragile cell count in pneumonia* A rise after the injection of [illegible] by a sharp drop. If the treatment *was beneficial the fragile c*[illegible] *failed, the fragile cells did not fall* below the danger zone and [illegible] p[illegible] as in those that recovered) they *rose very rapidly to their former high level.*

Fig. 6.—Group 6. Cases of lobar *pneumonia* and miscellaneous septic infections. At the time of complications the number of fragile cells increased The result of either surgery or specific measures, if favorable, was marked by a drop in the fragile cells

Fig. 7.—Group 7 Patients with lobar pneumonia who died All showed a high fragility at time of death; sometimes the fragile cells were low in the early stages but a gradual increase was observed as the patient became worse

Fig. 8.—Group 8. Cases of influenzal pneumonia All patients recovered The fragile cells were low.

Fig. 9.—Group 9. Pneumonia patients died with a low fragility Unfortunately some have but one count. No. 1 died of acute cardiac dilatation

Fig. 10.—Group 10. Two patients with lobar pneumonia who recovered with a high fragile cell count. We have no explanation for the apparent contradictions.

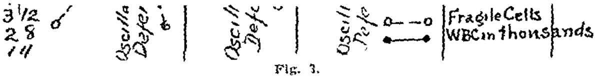


Fig. 3.

The principle of the method is as follows: the blood cells are subjected to a standard-injuring medium in the form of a hypotonic sodium chloride and sodium citrate solution. Then the blood suspension is brought to isotonicity by the addition of hypertonic saline solution and a nonvital dye is added.

With Sampson's method the differentiation between a fragile cell and a resistant cell is very sharp. The nuclei of the fragile cells appear diffusely colored by the dye and often these fragile cells are distorted. The resistant

cells remain colorless and refractile; the erythrocytes are largely removed by laking. Sampson considers that the percentage of fragile cells in the normal individual, is about 45 to 55 per cent (with 43 to 63 per cent as the extremes). He also studied the fragility of the leucocytes in several diseases.

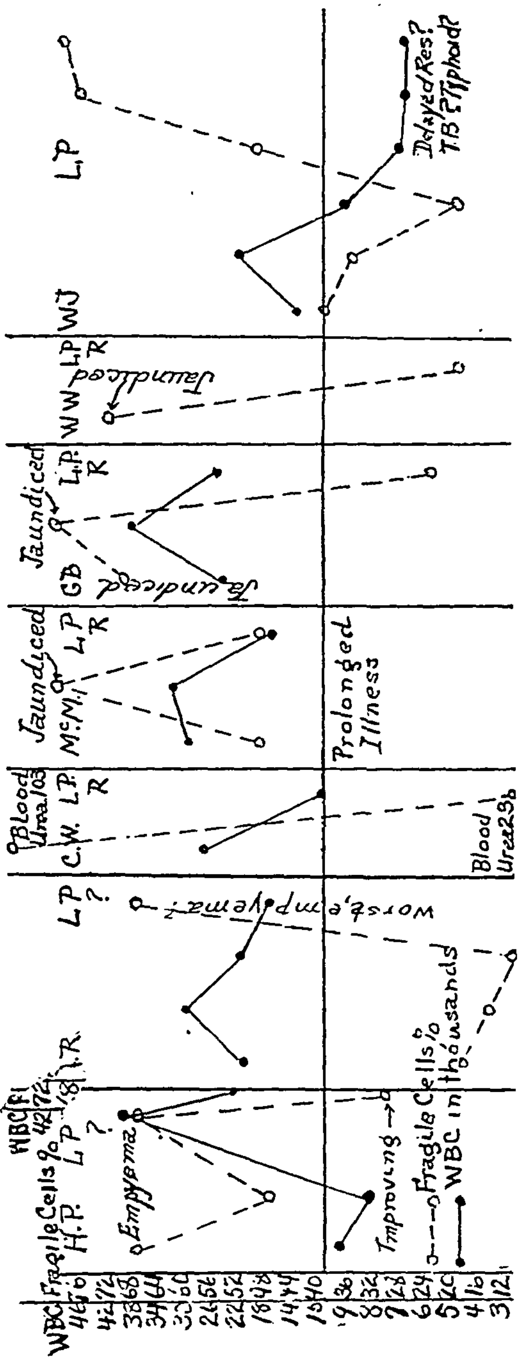


Fig. 4.

Of six patients with lobar pneumonia that he studied, four that recovered, showed a marked reduction of fragile cells and two that died, showed only a moderate reduction.

Pneumonia seemed a particularly suitable disease for this study; we were especially interested in noting the behavior of the fragile cells during its course. An effort was made to obtain as many counts as possible, especially at or near the crisis, and during convalescence. We have tried to correlate the influence of complications, treatment, and other factors which might

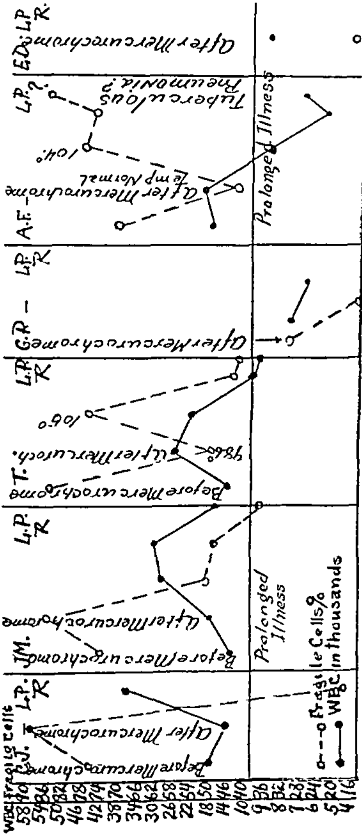


Fig. 5.

alter the course of the infection, and as a check on our technic, a few other acute infections were studied.

Method.—A drop of blood is drawn into a leucocyte counting pipette to the mark "1," and the diluting fluid, consisting of 0.1 gm. of sodium citrate and 0.05 gm. sodium chloride to each hundred cubic centimeters of distilled water, is drawn up to the "11" mark. The pipette is gently rotated while

the fluid is drawn up. It is not shaken, but slowly rotated while the mixture is blown into a test tube, of from 5 to 8 mm. internal diameter and from 3 to 6 cm. in length, which preferably has been lined with paraffin. The test tube containing the diluted blood is corked, preventing evaporation which would cause an alteration of the concentration, and is then gently agitated for one minute to insure an even distribution of the cells throughout the solution. It is then allowed to rest undisturbed for exactly two minutes more, during which period the same pipette is washed out with water and the barrel refilled to the point "1" with 9 per cent sodium chloride solution. At the expiration of the total three minutes the 9 per cent sodium chloride is blown into the solution, the entire mixture drawn once into the pipette and then expelled into the test tube. One drop of 1.0 per cent aqueous solution Niagara blue or 1.0 per cent trypan blue is then added to the contents of the tube, which is gently shaken once or twice and then recorked. A

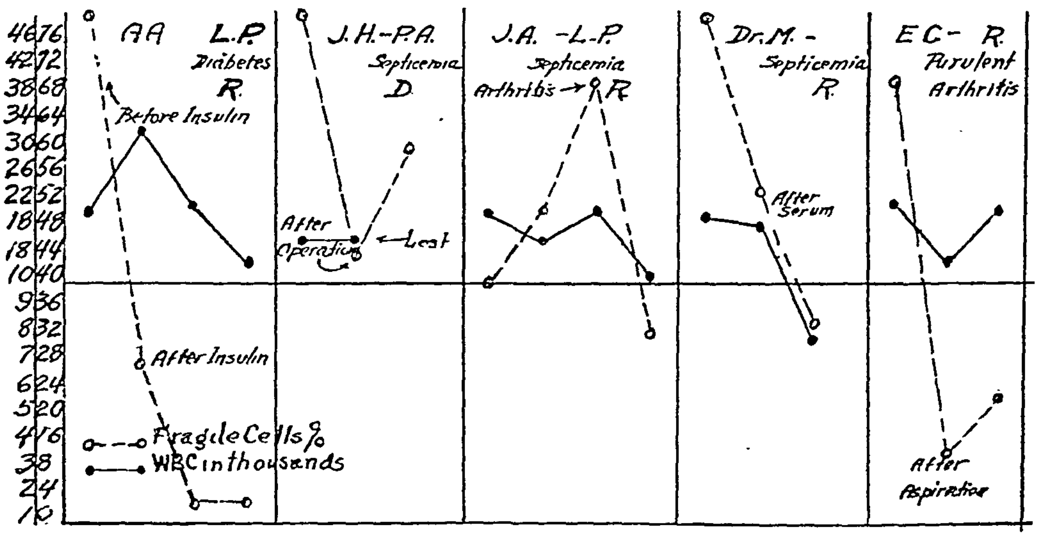


Fig. 6.

large drop of the solution is taken from the test tube, placed on a smooth glass slide and covered with a No. 0 or 1 thickness cover slip which should not contain marked ridges or other irregularities and should measure from 1.5 by 1.5 cm. to 2.5 by 2.5 cm. The preparation is then rimmed with melted paraffin. The relative number of stained and unstained granular and non-granular white cells is counted in the proximal, central, and distal portions of the preparation, running from one margin to the other of the cover slip.

Certain precautions need to be emphasized. Excessive trauma in collecting and manipulating the specimen should be avoided; the entire procedure should not consume more than fifteen minutes and therefore should be done at the bedside. While a dye of low toxicity should be used, such as trypan blue or Niagara blue, *vital* stains should be avoided, as these also penetrate the still living or resistant cell. The diluting solutions have to be changed from time to time and should be kept in the ice chest and free from gross bacterial contamination.

The following experiments convinced us that under the conditions of study, the stain or fragile cells are either injured or dead cells.

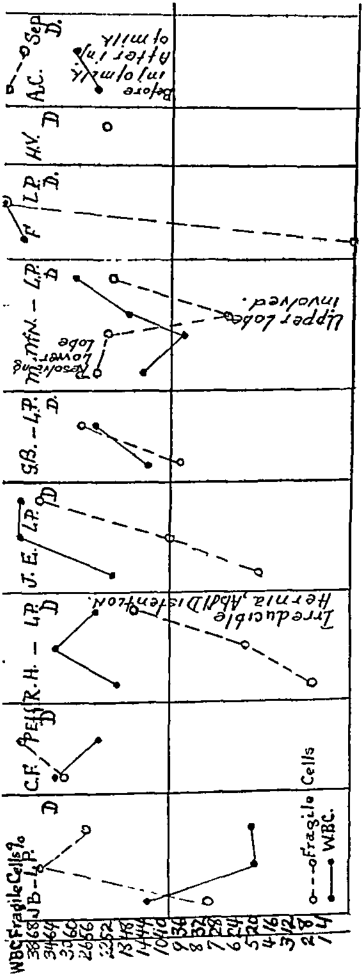


Fig. 7

Experiment No. 1.—

Spinal fluid from a case of purulent meningitis showed:

a. Before boiling—10 per cent fragile cells.

b. After boiling—91 per cent fragile cells.

It is safe to assume that the effect of heating the spinal fluid to the boiling point is to kill all the cells. The rise of fragile cells after boiling justifies the assumption that the so-called fragile cells are injured or dead cells.

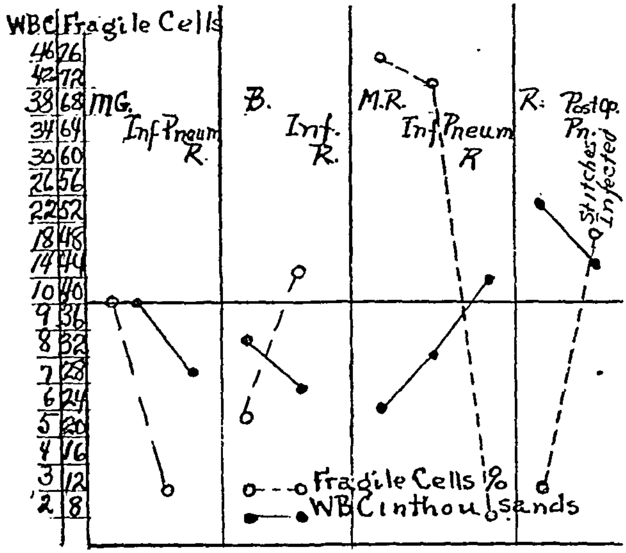


Fig. 8.

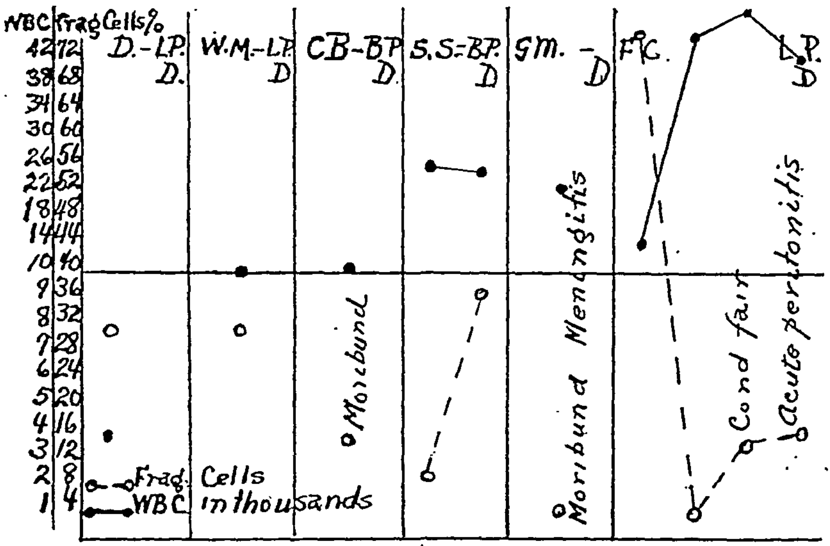


Fig. 9.

Experiment No. 2.—

Pus from a knee-joint showed 12 per cent fragile cells five hours after removal; twenty-four hours later, there were 36 per cent fragile cells; forty-eight hours showed 64 per cent and there were 88 per cent in seventy-two hours. At this time there was so much disintegration that it was difficult to separate the cells. The increase in the percentage of fragile cells was therefore parallel with the increase in cell death.

Experiment No. 3.—

The opsonic index done at the forty-eight-hour period showed that 68 per cent of the cells were no longer phagocytic. This figure corresponded very closely with the 64 per cent fragile cells for that period.

Experiment No. 4.—

The motility of the leucocytes was studied by the method of Florence Sabin. Distinct motion was observed in some cells at the end of forty-eight hours, corresponding with the opsonic index and the percentage of fragile cells for that time.

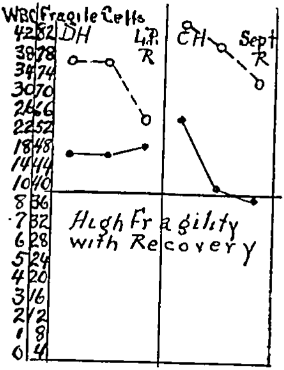


Fig. 10.

In recording our results with this test in pneumonia patients, we have arbitrarily called anything above 40 per cent fragile cells within the danger zone. The results obtained with this test, we hope to make clear by the accompanying charts.

SUMMARY

We believe that the study of the fragility of the leucocytes is of interest and value in the course of some acute infectious diseases. Like many other tests its findings are not *always* conclusive. It should never be considered by itself but along with other studies.

It has been observed that the fragile cells increase prior to complications; that a high fragility is almost always associated with either a stormy course or with a bad prognosis, particularly if accompanied by a normal or low leucocytic count. The curve of fragile cells does not depend on the leucocyte curve or necessarily correspond with subjective symptoms. In most cases this independence was gratifying, as it was a better indication of the outcome.

A single high fragile cell count is of little significance, especially in the beginning of the disease. Treatment or some other factor may change the curve of the fragility of the leucocytes, and the outcome also. We were

able to study the influence of mercurochrome in pneumonia. When it was beneficial there was a slight rise in the fragile cell count followed by a sharp fall; thereafter the percentage of fragile cells remained low. Other times, when mercurochrome was of temporary benefit only, there was a temporary tumble in the number of fragile cells; and thereafter the number of fragile cells increased rapidly. Drainage in infections, administration of sera in septicemias, of insulin in a lobar pneumonia complicated by diabetes, all produced a drop in the fragile cell count.

We must refrain from drawing any conclusions because our series is small—only fifty-five cases were studied—and our clinical observations have not been as complete as we would have liked to have had them. It seems necessary to determine the percentage of fragile cells within the limits of safety, for *each* condition in which the test is applicable. In this connection, for *pneumonia*, under our conditions, anything above 40 per cent is within the danger zone. Mauriac's "Oscillation of Defense" which was observed in a number of cases has been discussed elsewhere in this paper.

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[3]...mpson, John J.: Determination of the Resistance of Leucocytes, Arch. Int. Med., October, 1924, xxxiv, 490-502.

THE ORGANIZATION OF A METABOLISM LABORATORY*

By William H. Stoner, A.M., Ph.D., Philadelphia, Pa.

THE organization of a metabolism laboratory should be planned by the clinician and the laboratorian, preferably a biochemist, who are to have charge of the department of metabolism. Very rarely this dual combination of metabolic clinician and biochemist is found in one man. By study of the organizations of, and visits to, similar departments and by frequent conferences, they should draft and redraft their plans until a satisfactory one is evolved.

It is assumed that a hospital planning a department of metabolic diseases has thoroughly equipped and manned general laboratories of chemistry, pathology, bacteriology, serology, roentgenology, animal experimentation and necropsy, and that these services are available to the metabolism department for the usual routine examinations and experiments.

In addition to these general laboratories there should be established, in an efficient department of metabolic diseases, a departmental laboratory—chiefly chemical and research—which should be so equipped and manned as to be able to supply any service requested by the clinical departmental staff, including emergency service at any time of the day or night,—week days, Sundays, and holidays. If the general laboratory of the hospital does not include departments of physics, physiology, electrocardiography, x-ray, glass-blowing, and machine shop, they must be provided for in the departmental plan.

On account of the emergency nature of a great portion of the work of a metabolic laboratory, it is quite important that the laboratory be located conveniently near the clinical department, certainly under the same roof and preferably in an adjoining room. Blood and urine sugar and plasma CO_2 reports should be forthcoming in emergency cases in fifteen to twenty minutes after taking samples.

The size of the laboratory depends upon the size of the clinical department. The usual fault in planning such a laboratory is to allow too small, rather than too large, a space. Usually within a very few years after the establishment of such a laboratory it is found to be inadequate in size, equipment, and personnel. For the usual routine work conducted by the less trained members of the staff, one large, airy, light room should be provided. For the chemists, research clinicians, food analysts, gas analysts, etc., specially equipped, more private, and smaller rooms should be available. At least two or three rooms should be fitted for general research work for additional workers not initially contemplated. Special provision should be made

*Read by invitation before the Fourth Annual Convention of the American Society of Clinical Pathologists in Philadelphia, May 20, 1925.

for instructional purposes since instruction in all laboratory matters, of nurses, patients, visiting students and internes should be conducted in the laboratory. Such arrangements should include a suitable assembly room equipped with blackboards and demonstration table, thoroughly equipped with gas, hot and cold water, drain, blast, vacuum and electricity. Charts, diagrams, models, etc., should be available. Office space should be provided for the conduct of records and the preparation of manuscripts.

In charge of all administrative matters and of routine and research laboratory work should be a biochemist, Ph.D., M.D., or both, thoroughly trained in general physiology, including animal experimentation, physiologic chemistry, physics, mathematics, German and French, and having considerable experience in practical hospital routine laboratory work, especially blood and urine chemistry. He should also have had a thorough training in food analysis and in food and animal calorimetry.

Under the direction of the biochemist should be one or more junior chemists of similar but less complete training and experience, who should perform the analyses suggested by the biochemist in the departmental researches.

In addition to the biochemist and his chemically trained assistants there should be provided one or more technical assistants for the performance of the more frequent determinations. In regard to the nature of the training of these technical assistants, there is much difference of opinion. Experience shows that the less training they have had in chemistry, physics, or medicine the better technical experts they become. Many regularly trained laboratory technicians consider their superficial training an adequate one for the more advanced work, even the research work of those in higher stations in the personnel. The best routine chemical work is done by high school boys with no technical training except in the very particular tests for which they are responsible. This necessitates rigid supervision by a chemist of the solutions used in the determinations and of the results obtained.

In addition to the personnel outlined, one or more clerks should be provided so that the more valuable time of the chemists is not lost in longhand writing of their results for publication or the time of the technicians lost in the cataloging and filing of results. In general, in an organization of any sort the most economical practice is to assign duties to the lowest paid member of the staff who can properly perform them. For this reason it is well to have at least one member of the personnel an unskilled laborer, and it is surprising how much of the less complicated operations of a laboratory can be performed by this employee. In the same way, after some time, the technician although formerly untrained is able to prepare the solutions used and to recognize defects arising in the work which at first required the services of one of the chemists.

There are two common errors in the initial equipment of laboratories, deficiency and excess. With the usual hospital appropriation for this purpose, however, the error is usually in the former direction. It is quite rare to see a hospital laboratory equipped with expensive dust covered apparatus which has never been used. On the other hand, it is quite common to see a laboratory unequipped, or poorly equipped, for very necessary diagnostic

work. The most expensive is usually the least extravagant. An example of this in a biochemistry laboratory is colorimeters. The cheaper, mechanically poorer colorimeters are in the end much more expensive than those of higher initial cost. The same is true of the ordinary glassware as compared with the resistance types. Again, it is no economy to use a cheap chemical when results obtained will be doubtful on that account. A laboratory for the study of metabolic diseases should be equipped to perform all the usual chemical tests of the urine, blood, feces, gastric juice and other body fluids. It should also be equipped for complete food analysis of any sort including bomb calorimetry and for all the usual respiratory exchange work used in determining respiratory quotient, basal metabolic rate, etc. All the modern functional tests should be provided for, including those of the liver, kidney, stomach, etc.

The equipment should include a library containing a small but well-selected group of textbooks and journals. The medical literature abounds in instances of research work duplicated after a period of years without the knowledge of the later author. Research work in any line should be preceded by complete bibliographic research. The departmental libraries of laboratories in more remote sections should be larger than in the cities having large medical libraries.

The biochemist should be the executive head, responsible in line of authority to the chief of the Department of Metabolic Diseases of the hospital. He should be responsible for the assignment of duties to the various members of his staff. He should control the purchase and use of supplies and reagents, making recommendations for changes in personnel, and should direct the entire research of the department, clinical as well as chemical. He should establish and maintain a routine statistical research program by a complete system of records of all analyses of the department. The difficulty usually encountered in the assignment of executive duties to a scientific departmental head is that he becomes sterile scientifically and devotes too great a proportion of his time to executive duties. With proper systematization and assignment of these executive responsibilities to other members of his staff he should be able to devote at least three-quarters of his time to investigative work.

In the conduct of an out-patient department in diabetic work, it is necessary to have immediate laboratory service so that blood and urine analyses may be conducted while the patient is in the dispensary. For this reason it is essential that at least one technician be assigned to the dispensary for the analyses of samples taken at that time. Dispensary hour should be early in the morning so that blood samples may be taken before breakfast, and so that the occupations of the out-patients will be interfered with as little as possible. With such a service the clinician can prescribe dietary and insulin treatment with almost mathematical precision.

A metabolism laboratory with an organization as outlined is able not only to render adequate and prompt diagnostic and therapeutic service but also to contribute much to the knowledge of the practically unexplored field of metabolic diseases.

EXPERIMENTAL AND CLINICAL OBSERVATIONS ON THE ACTIONS AND THERAPEUTIC USES OF ETHYLISOPROPYLBARBITURIC ACID*

By D. E. Jackson, Ph.D., M.D., and Louis A. Lurie, M.A., M.D., Cincinnati, Ohio

IN a preliminary article[1] published by one of us in 1922, it was shown experimentally that a new chemical compound, ethylisopropylbarbituric acid, which had just been synthesized in the laboratory by Lambert Thorp, possessed hypnotic properties which apparently gave marked promise for the future usefulness of the compound in medical practice. Following up this experimental work a short account of the early clinical application of the drug was published in 1923.[2] Chemically, the compound is made by condensation of urea and ethylisopropyl-malonic-diethylester in the presence of sodium ethylate at 105° C. The free acid is but slightly soluble in cold water, but its sodium and potassium salts are freely soluble both in water and in alcohol.

Recently Thorp has prepared the calcium salt of this compound. Encouraged by the favorable results of our earlier work with the substance we have continued our investigations, using more recently this newly prepared calcium salt. This is a white, sandlike powder which is sufficiently soluble in water to permit of its experimental use by intravenous injections in animals.

In order to determine its action on the respiration and circulation, we have carried out a series of experiments on dogs, the results of which have been very satisfactory. The accompanying tracings show this as may be seen by reference to Figs. 1, 2, and 3.

In Fig. 1 we have shown the action of 3 c.c. of 1.5 per cent solution on the blood pressure (mercury manometer) and respiration of a dog weighing 11 kilos. This represents a dose of about ¾ grain (45 milligrams) or the equivalent of a medicinal dose (3.5 to 4 gr.) in man. The drug here, however, was all suddenly injected into the blood by way of the femoral vein. In this case the greatest toxic action which this quantity of the drug can produce is shown immediately on the blood pressure and respiration. It is seen that the arterial pressure is at first slightly increased (as a result of the volume of the injected solution only) and that this is followed by a small, transient fall in pressure which lasts only about forty seconds, by the end of which time the blood pressure has again returned to practically its previous normal level. This shows that doses of this size have but little action on the circulatory system, and that, therefore, one would not expect any serious symptoms to arise from the circulatory organs in the ordinary clinical use of the drug.

*From the Medical Department, University of Cincinnati, and the Psychopathic Institute of the Jewish Hospital, Cincinnati, Ohio.
Received for publication, June 12, 1925.

Fig. 1 further shows that the dose here used has almost no effect whatever on the respiration. The rate remains approximately constant while the variations in depth are so small as to be practically negligible.

Fig. 2 shows the results of two injections (3 c.c. and 7 c.c.) on the heart and on the blood pressure. In this experiment the chest was opened under ether anesthesia and a myocardiograph was attached directly to the heart. The record obtained from the heart is shown in the upper broad tracing in which the downstroke of the recording tambour represents the systole of the heart. The blood pressure (right carotid artery) was recorded with a mercury manometer. Here it is seen that 3 c.c. of 1.5 per cent solution of

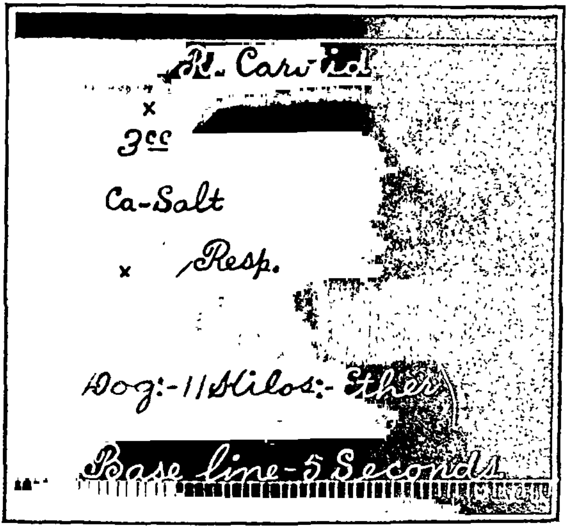

Fig. 1.

the drug injected at the point indicated by X on the tracing had but little effect on the heart, the extent of both systole and diastole remained almost unchanged. A slight fall in blood pressure was produced but in less than two minutes the pressure had again regained its normal level. Following the first injection of 3 c.c., a large injection (7 c.c.) was given. This caused a very pronounced fall in blood pressure, but recovery from it was good and in about three minutes the pressure had again risen to normal. The extent of both systole and diastole of the heart was slightly reduced, but within a few minutes the beat had again returned to approximately its normal extent and vigor. Here we see that within a space of about four minutes no less than 150 milligrams (3 c.c. and 7 c.c. of 1.5 per cent solution) of the drug were thrown directly into the circulation and yet in less than three minutes

after the last injection the heartbeat had returned to very nearly its normal amplitude and strength while the blood pressure was fully back to normal. This experiment shows that the drug has but little deleterious action on the heart muscle itself or on the circulatory apparatus in general when the substance is given in quantities more than four times as large as the ordinary medicinal dose (3.5 to 4 gr.) by stomach; and when taken by stomach, of course, the drug would pass into the circulation very much more slowly than was the case in this experiment in which the drug was injected suddenly into the femoral vein.

In Fig. 3 the remarkable recovery which the heart and circulation in general can make after enormous quantities of this drug is well shown. Here the upper tracing (myocardiogram) was taken directly from the heart itself while the blood pressure was recorded by a mercury manometer. A total of 6 grains was injected intravenously into an animal weighing 14 kilos. The drug produced a striking effect on the strength and amplitude of the heartbeat, and the blood pressure fell sharply, yet after about five minutes the heartbeat had almost returned to normal and the blood pressure was only a few millimeters below its normal level.

In other experiments it has been shown that very large doses of the drug may be injected intravenously without causing the death of the animal,* but that when death does occur it is due to central respiratory paralysis. Consequently the treatment for poisoning with the substance should consist of respiratory stimulation or artificial respiration.

When the drug is administered in therapeutic doses the action is manifested almost entirely on the central nervous system and more especially on the cerebrum. In animals, there appears to be produced an overpowering desire to lie down quietly and sleep. After moderate doses a dog can be awakened by loud sounds, shaking, etc., but if the animal is then left alone it soon lies down and goes to sleep again.

In animals, large doses may produce slight muscular twitchings or tremors. From a practical standpoint these appear to be of no importance as they do not appear in man following the therapeutic administration of the drug.

From the therapeutic standpoint, the ideal hypnotic should be a pure chemical compound, fairly soluble in cold water, of not unpleasant taste and possessed of a rapid action. The dosage should be small with a wide latitude between the therapeutic and lethal doses. It should be free from immunizing effect, should not color the urine—an indication of destruction of the red blood corpuscles—and it should be free from a depressing action

*From the therapeutic action of the drug it seems obvious that man is more susceptible to the hypnotic effects of the compound than are dogs. In the earlier reported experiments with the sodium salt on dogs, it was found that the dose (by mouth) beyond which recovery does not occur is in the neighborhood of one grain to the pound of weight of the animal. This would indicate that an average patient of one hundred and fifty pounds weight might survive a dose of nearly ten grams or one hundred and fifty grains. It seems probable however, that the fatal dose in man would be smaller than this amount, unless the stomach should be emptied very soon after the drug had been swallowed. The lower degree of susceptibility possessed by dogs, as compared with man, probably holds only in case of the higher, psychical areas of the cerebrum; and in this case, man's higher psychical development probably mainly accounts for his increased susceptibility over that of the dog. So far as the heart and the other circulatory organs and the respiratory apparatus are concerned, it is probable that no great difference exists between man and the dog.

on the heart. Finally, it should not cause the patient to have a feeling of dopiness or drowsiness upon awakening. Moreover, its action should be selective, only the highest cerebral cells being affected by even large doses.

Such a hypnotic we feel is represented by ethylisopropylbarbituric acid. This substance is a white crystalline compound slightly soluble in cold water. Its taste is not disagreeable and its action is very rapid. It has been used by many physicians in a great variety of cases, and so far no untoward effects upon the heart, lungs, or kidneys have been noted. The outstanding feature in all cases is the ease with which a refreshing sleep is produced and without the usual feeling of lassitude and torpor on the following day. In many instances sleep was produced where other well-known hypnotics had

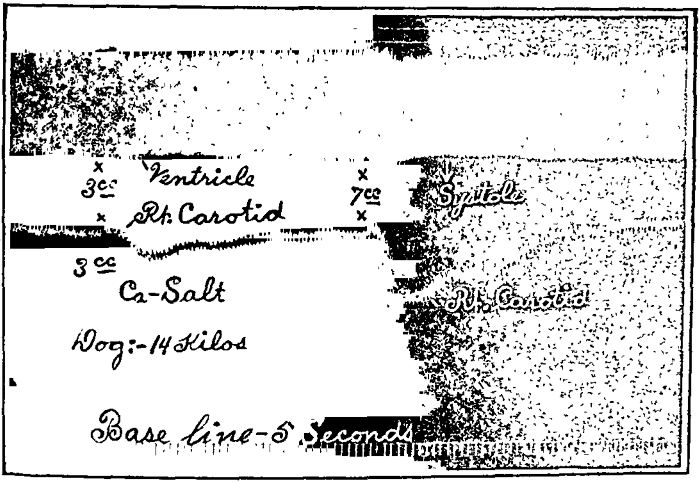

Fig. 2.

failed. The dosage has been from three and one-half to four grains, although as much as fifteen grains may be given with absolute safety. The drug has also been given hypodermically to maniacal individuals with marked sedative effect. Many surgeons have found it of particular value in combating the motor restlessness, irritability, and sleeplessness so often met with in postoperative cases.

Many individuals who are operated upon are found to have more or less impairment of the myocardium. Many of these patients require and receive hypnotics and this despite the fact that hypnotics, as a rule, are known cardiac depressants. Obviously, in such cases the choice of a hypnotic is of the greatest importance. A large number of patients all of whom showed definite myocardial involvement, and on whom various major operations had been performed, were given this drug and the effect on the heart and pulse

carefully noted. In every case without exception a refreshing sleep was obtained with no deleterious action on the heart or pulse.

Sleeplessness as a symptom is met with in a great variety of conditions, both functional and organic. Every physician knows what a troublesome symptom it is and how quickly a patient may come to rely absolutely on the use of hypnotics to produce sleep. Unfortunately, most hypnotics lose their effect quickly and the dosage must be increased. The chronic sufferers are, therefore, forced to change from one hypnotic to another irrespective of their value or danger.

Ethylisopropylbarbituric acid has been given to a great many individuals for the past three years, and so far it has been found necessary to increase

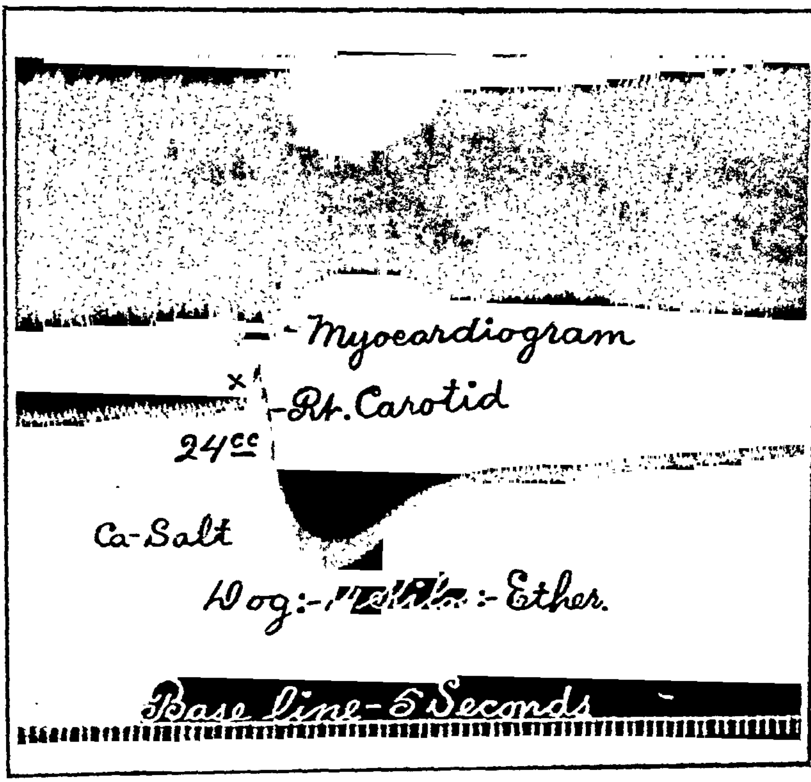

Fig. 3.

the dose from three and one-half to six grains in but very few cases. In this connection the following case report is of interest:

J. J., age fifty-eight years, was under observation at a hospital suffering from an anxiety state, the principal symptom of which was the fear of "contamination". The man was in a fearfully nervous state and suffering terrific mental anguish. He felt that even touching his bedclothes and nightgown "contaminated" him. For about ten days previous to his admission to the hospital he had been unable to sleep. Mild sedatives and hydrotherapeutic measures were tried, but without result. He was thereupon given a nightly dose of three and one-half grains of the calcium salt of ethylisopropylbarbituric acid. During the several months that the patient was in the hospital he never failed to obtain a refreshing sleep. On several occasions five grains of veronal were given instead of the three and one-half grains of ethylisopropylbarbituric acid. The patient, however, obtained very little sleep. Ten grains of veronal were then given. The difference in the

sleep produced by ten grains of veronal and three and one-half grains of the calcium salt of ethylisopropylbarbituric acid was marked. True, the patient slept under the ten grains of veronal but it was a heavy, troubled sleep which left him dopey and drowsy the next day, and hard to control. After the exhibition of three and one-half grains of the calcium salt of ethylisopropylbarbituric acid, the sleep approached closely that of a normal sleep and the patient felt refreshed on awakening and showed no mental lassitude or torpor.

This hypnotic has also been of great value in curing individuals of the habit of taking hypnotics. This habit is prevalent among the group of patients diagnosed as neurasthenics and psychasthenics.

The effect of the older hypnotics is normally of a fleeting nature; sleep of a greater or lesser degree being obtained for one night only. The following night the neurotic individual must either take another dose or go sleepless. Furthermore, as pointed out above, in a short while the drug loses its effect unless the dosage is doubled or trebled. The harmful effects of such a procedure can be readily inferred. In addition, any individual who is a slave to a habit, no matter what its nature, suffers tremendously in one respect, namely, in loss of self-confidence in regard to that particular habit. No habit can be cured until the patient's self-confidence is restored to such an extent that he is made to feel that he is master of the habit which has enslaved him.

One of the characteristics of this new hypnotic is the fact that it not only produces sleep the night that it is exhibited but its sedative action persists so that as a rule the patient will also be able to sleep the following night without resorting to the drug. A patient who had previously found it necessary night after night to resort to hypnotics is not only greatly improved but highly elated when he finds that he is able to do without a hypnotic even for one night. This furnishes the wedge by which the patient can be convinced that he can ultimately dispense with hypnotics.

Many case reports bearing this out could be cited. However, one will suffice.

H. A., aged sixty-two, came under observation because of a severe mental depression. The history revealed that at the age of twenty-five, a partial thyroidectomy had been performed following which she became a chronic invalid. Of recent years she has been greatly troubled with insomnia and had to resort to the nightly use of veronal. Lately she has found that five grains did not produce sleep and she has had to double the dose. Three and one-half grains of the calcium salt of ethylisopropylbarbituric acid were substituted for the veronal and little by little the patient was gradually weaned away from the use of all hypnotics. The result in this case can be attributed principally to the fact that this new hypnotic not only produced a natural sleep the night the drug was taken, but also had a distinct effect the following night. Once the patient discovered that she could sleep without resorting to hypnotics, her self-confidence was reestablished and her cooperation was assured. The desired result followed naturally.

In conclusion, we wish to call attention to the fact that this new hypnotic in laboratory as well as in clinical tests, has shown superiority over the older hypnotics, both in safety and in effectiveness. The margin of safety between the therapeutic dose and lethal dose is very great. Furthermore, no untoward effect on the heart, lungs, or kidneys has been observed. In addition, it produces a sleep which approximates normal sleep very closely

and without the usual feeling of lassitude and heaviness of the following day, a condition that is so characteristic of other hypnotics.

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THE EFFECT OF HEAT ON THE HEART EXTRACT USED AS ANTIGEN IN THE WASSERMANN TEST FOR SYPHILIS*

By S. L. Leiboff, New York

SINCE 1906, when Wassermann, Neisser and Bruck[1] pointed out the possibility of making use of the phenomenon of complement fixation discovered by Bordet and Gengou[2] in the diagnosis of syphilis, the problem of producing a suitable antigen has occupied the minds of many workers. It was very natural for Wassermann to use as antigen tissue extracts of a congenitally syphilitic fetus, since he considered the complement-fixation reaction in syphilis a true antigen-antibody reaction.

This theory of a specific antigen-antibody was soon disproved by Marie and Levaditi[3] who obtained positive reactions in paresis with an aqueous extract of normal liver. New light was thrown upon the subject by Landsteiner, Müller and Potzl,[4] who demonstrated that just as good results could be obtained with alcoholic extracts of guinea-pig heart as antigen as when aqueous extracts of fetal syphilitic liver were employed. Porges and Myer[5] thought that the active principle of the syphilitic antigen was lecithin. Browning, Cruickshank and McKenzie[6] found that the addition of a certain amount of cholesterol to alcoholic antigens greatly increased their antigenic value. Levaditi and Yamanuchi[7] showed that cholesterol alone has no antigenic properties.

Our knowledge was further increased by Noguchi,[8] who classified the lipoids which act as antigens, according to their solubilities in the ordinary fat solvents; alcohol, ether and acetone. He found that the active principle in the antigen is acetone-insoluble, thus proving it to be a phosphatide. He also formulated a system of titration for judging the value of an antigen.[9] He set down the following rules which must be strictly adhered to in order to obtain good results: The greater the dose of antigen used for obtaining a positive reaction with a known positive serum, the greater are the chances of picking up a doubtful reaction; at least five times the dose of antigen necessary to inhibit hemolysis with a known positive serum should be used in performing the Wassermann test. This dose must not be hemolytic by itself, and must not inhibit hemolysis with negative sera. Thus in titrating an

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antigen we must learn its three essential properties: antigenic, hemolytic and anticomplementary.

There are three kinds of antigen in general use at present: the plain alcoholic extract, the alcoholic extract with the addition of cholesterol, and the Noguchi acetone-insoluble antigen. Shuermann,[10] Sachs and Rondoni[11] used synthetic antigens which contained lecithin as a basis, and although some success was obtained with some of them, the results were not good enough to warrant their use in performing the Wassermann test. Good results, however, are obtained with the three antigens mentioned above, provided they are properly titrated.

Most laboratories make use of three different antigens as it seems unsafe to depend on the results obtained with a single antigen. The plain alcoholic extracts are the weakest antigens, and while they give no false positive results yet they may fail to pick up weakly positive sera, and it is these borderline cases which are of great importance. The cholesterinized plain extracts are the strongest antigens but may sometimes give false positives. The acetone-insoluble antigen occupies an intermediate position and is probably the safest to use. However it is not used much because of the difficulties encountered in its preparation.

The technic for preparing plain alcoholic and cholesterinized antigens is quite simple and yet it is difficult to procure usable antigens; by using the very same method one may obtain an excellent antigen from one heart while the next dozen hearts may give worthless extracts.

In order to overcome this difficulty, and to evolve a method for obtaining a good antigen every time, Neymann and Gager[12] investigated the lipoids of heart-muscle and classified them according to their antigenic value in the Wassermann test. They extracted the various fractions of lipoids, following the method of Erlandsen[13] which divides the various lipoids according to their solubilities in ether, alcohol and acetone. In the main, their method consisted in making two extracts, a primary ether-soluble and a secondary alcohol-soluble extract. This method was chosen because only 15 to 30 per cent of the total fats and lipoids present in tissues are obtained on exhaustive extraction with ether. The remainder is easily extracted with alcohol. This observation was first made by Hoppe Seyler[14] and was later confirmed by Erlandsen,[13] Cohn,[15] and others. As to the nature of these two fractions of lipoid material there is a diversity of opinion; thus Erlandsen believes that the ether and alcohol soluble fractions contain different lipoids, while MacLean[16] claims that the two lipoids are the same. He attempts to explain this phenomenon on the supposition that the ether fails to penetrate the tissues, the difficulty being a mechanical one. The work of Neymann and Gager, however, favors the view held by Erlandsen, since the ether and alcoholic fractions show different antigenic properties. They recommend a method for making up antigen, for which they claim superior qualities over antigens prepared by the ordinary methods. Their method is as follows: After removing as much as possible of the endocardium and pericardium, and the larger blood vessels and fat from a normal beef-heart, the muscle is ground very fine, spread in a thin layer on glass plates and dried by means of an electric

fan for twenty-four hours. The dry tissue is ground up again to a fine powder, placed in cloth bags and dried in an incubator at 37° C. for several days. The powder is then extracted with ether in a Soxhlet extraction apparatus for twelve hours, or in an ordinary wide-mouthed bottle at room temperature five or six times until the supernatant fluid is no longer colored yellow. This takes from eight to ten days. The ether-free powder is now dried and extracted with 95 per cent alcohol for ten days. It is then filtered and is ready for use.

The authors claim to have prepared ten antigens successively from ten different hearts obtaining equally good results. Their antigens have a binding power of 1 to 1600, low anticomplementary titer, and no hemolytic properties.

I have prepared three different antigens from three different beef-hearts according to the above described method and verified the claims of Neymann and Gager. The two important points established by these authors are, that tissues used in preparation of antigens for the Wassermann test can withstand drying at room temperature; and that by first removing the ether-soluble lipoids from the tissues a better antigen is obtained. This method, however, while having the advantage over the older methods in that better antigens are obtained, yet the method of preparation is very tedious and requires from two to three weeks to prepare an antigen.

Noguchi[17] divided the extracts of normal tissue into four groups as follows:

Group I.—Insoluble in ether and hot alcohol. Consists chiefly of proteins and salts. Proteins bind complement with certain active sera; this binding is due to some other substances present in the serum not identical with the one that binds complement in the Wassermann test. Thus the presence of protein in an antigen, by its fixation of complement, would tend to obscure the Wassermann test and give false positive results with nonsyphilitic sera.

Group II.—Insoluble in ether but soluble in hot alcohol. These consist of soaps, cleavage products of proteins, and bile salts. Soaps and bile salts are strongly hemolytic and very slightly antigenic.

Group III.—Soluble in ether, alcohol and acetone. Contains saturated fatty acids, cholesterins, lipoids of unknown nature. These substances are anticomplementary or [illegible]olytic.

[illegible]oup IV.—Inso[illegible] acetone but soluble in ether and alcohol. These [illegible]sphatides, am[illegible] [illegible]ch lecithin is the best known.

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Group III.—Soluble in ether, alcohol and acetone. Contains saturated fatty acids, cholesterins, lipoids of unknown nature. These substances are anticomplementary or hemolytic.

Group IV.—Insoluble in acetone but soluble in ether and alcohol. These are phosphatides, among which lecithin is the best known.

Thus Noguchi prepared his well-known acetone-insoluble antigen by extracting organs with alcohol for about ten days to two weeks at 37° C. or for longer periods at room temperature. The alcohol is then evaporated at room temperature, the residue taken up with ether and precipitated with acetone.

No matter what method is used in preparing an antigen, upon one point all workers have agreed, and that is that all extractions must be done at temperatures not above 37° C. The reason for such precaution is very obvious since it was proved that the binding component of the antigen consists of "lecithin-like" substances, and certain of these substances are of a very labile nature; too long exposure to the oxygen of the air or high temperature

greatly increases the ease with which oxidation of their unsaturated fatty acids takes place. The usual method[18] for obtaining phosphatides is to get the tissues dried in the shortest possible time with a minimum exposure to air at the lowest temperature that can conveniently be employed.

In preparing substances of lipoidal nature great care was always taken to remove as much water as possible from the tissue previous to applying a solvent. This is necessary because the solubilities of the various lipoids in alcohol and ether vary with the amount of water present. There are also indications that phosphorus-containing substances other than lipoids are extracted along with the phosphatides in the presence of even minute quantities of water.[18] It was for this reason that Collison[19] suggested that solvents used for lipoid extraction should be water-free.

Noguchi improved the antigen by precipitating with acetone, i.e., by removing the acetone-soluble substances. Neymann and Gager improved it by first drying the tissue and then removing the ether-soluble substances Taking these facts into consideration I thought that by combining the two methods, that is, by first drying the tissue, then extracting with ether, followed by an extraction with acetone, thereby removing from the tissue both the ether and acetone-soluble substances, a good antigen ought to be obtained. If in addition, the drying and extractions could be done at high temperatures, the time consumed in the process would be greatly shortened and a good antigen would be produced in a short time. It is true that most of the lipoids are thermolabile; however, this is not true of all of them. The diaminomonophosphatides, for example, contain no unsaturated fatty acids and are heat-stable. Noguchi[17] states that there seems to be a certain relationship between the number of unsaturated bonds, as determined by the iodine number, and the binding power of an antigen. The work of Neymann and Gager, however, seems to oppose this view by showing some evidence that the active principle in the antigen is a phosphatide belonging to the group of diaminomonophosphatides, which was shown by Levene[20] and others to contain no unsaturated fatty acids.

PROCEDURE

Six fresh beef-hearts were ground up in a meat grinder. A small portion was placed in ten times its weight of alcohol and kept at 37° C. to be used as a control. The remainder of the tissue was divided in two equal portions; one part was spread in thin layers over large glass plates and dried under an electric fan. It took thirty-six hours to get the tissue dry. The other portion was dried on a boiling water-bath. The dried tissues were then ground to a fine powder and each divided into four portions which were treated as follows:

1. Extracted with ether in Soxhlet for twenty-four hours.
2. Extracted with acetone.
3. Extracted with ether followed by extraction with acetone.
4. Taken up in absolute alcohol without any previous treatment.

The acetone extractions in the case of the heat-dried tissues were done in the Soxhlet extraction apparatus for twenty-four hours, while the tissues

dried at low temperature were extracted by pouring acetone over the tissues and keeping in the incubator at 37° C. with occasional shaking. These extractions continued for a week with daily changes of acetone.

All the samples after being dried were placed in absolute alcohol; the tissues prepared at low temperature were placed in the incubator at 37° C. and kept for seven days with occasional shaking. The tissues prepared at high temperatures were extracted with the alcohol on the water-bath at 70 to 75° C., just below the boiling point of alcohol, for two hours. The alcoholic extracts were then cooled and filtered through filter-paper.

All titrations were done a week later when the rest of the antigens were ready to be removed from the incubator. The proportion of tissue to alcohol was 1 gram of tissue to 10 c.c. of alcohol.

TITRATION OF ANTIGENS

One-fourth Wassermann quantities of an antisheep hemolytic system were used. The complement was taken from a pool of three or four guinea pigs and its dose was determined immediately before use. The doses used were two units of complement and two units of amboceptor.

0.25 c.c. of a 5 per cent sheep cell emulsion.
0.05 c.c. of syphilitic serum.
0.1 c.c. negative serum.

In order to obtain a syphilitic serum which just completely inhibits hemolysis, a strongly positive serum was first tested in a set of dilutions with saline, and the highest dilution which still gave complete inhibition of hemolysis was used in the test. The positive serum was inactivated for ten minutes at 56° C. The first incubation was done in the ice box for four hours, sensitized cells were then added and the second incubation was done in the water-bath at 37° C. for twenty minutes and the results read.

The antigens were made up in two dilutions: 1 to 10, and 1 to 40, by slowly adding saline from a pipette to a measured amount of antigen into a flask, shaking it gently all the while.

A series of fifteen tubes was set up, the first four tubes receiving amounts of the 1 to 4 diluted antigen, while all the remaining tubes contained increasing amounts of the 1 to 10 dilution.

TABLE I

	BINDING POWER						ANTICOMP. PROP.					HEMOLYTIC POWER			
Tube	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1/40 ant. c.c.	.01	.02	.05	.1											
1/10 ant. c.c.					.05	.1	.2	.5	.75	1.0	1.2	.5	1.0	1.0	1.0
Final Dil.	1/4000	1/2000	1/800	1/400	1/200	1/100	1/50	1/20	1/13	1/10	1/8	1/20	1/10	1/10	1/10

The first six tubes containing antigen in dilutions ranging from 1 to 4000 to 1 to 100 each contained 0.05 c.c. positive serum, thus serving as a test for the antigenic power of the antigens. Tubes 7 to 11 inclusive contained no serum (except Tube 7) and served to demonstrate the anticomplementary property. Tube 7 contained 0.1 c.c. of negative serum, thus showing the spec-

ificity of the antigens. The last four tubes indicate the hemolytic property. Tubes 12 and 13 contained no serum and no complement. Tube 14 contained no serum and no complement and no amboceptor.

Controls were also set up with positive and negative sera without antigen to make sure that the hemolytic system was right. In writing the results the following signs were used:

++++ = complete inhibition of hemolysis
+++ = slight hemolysis
++ = half inhibition
+ = slight inhibition
0 = complete hemolysis
+? = doubtful

The following points were brought out by the experiment as seen in Table II:

1. A preliminary ether extraction improves the antigenic and anticomplementary properties of an antigen. It also removes hemolytic components by removing free fatty acids, fats, etc.

2. Subsequent extraction with acetone rather impairs an antigen by diminishing its binding power, probably by removing antigenic lipoids.

3. The outstanding feature is the fact that the phosphatides responsible for the complement fixation in the Wassermann reaction can withstand high temperatures for considerable lengths of time. Heat not only does not injure them but seems to improve them.

This experiment was repeated twice and the same results were obtained each time. Accordingly the following method was used for preparing an antigen:

METHOD FOR PREPARING AN ANTIGEN

Fresh beef-heart is ground up in an ordinary meat grinder. It is then placed in a large flask with an excess of water and autoclaved for ten minutes or just boiled for about half an hour over a free flame. The tissue is then filtered through a cloth, such as a towel, and washed a few times with hot water. This process removes free fat and extractives. The tissue is then pressed to remove as much water as possible, and dried on the water-bath or in an oven at 100° C. until completely dry. It is then ground up finely and extracted completely with ether in the following manner: The powdered tissue is placed in a filter cup such as is used in the Soxhlet apparatus for fat extraction. The cup is suspended in a flask to which a reflux condenser is attached. The cup can easily be attached to the cork by thin copper wires passing through the cork.

The advantage of this method for extracting with ether is twofold; it saves a great deal of time and ether, the whole extraction is completed in three to four hours with a small amount of ether; and second, the ether used in this manner is practically anhydrous and devoid of impurities, since only the vapors of the ether come in contact with the tissue. When the ether extraction is complete, the powder is removed from the filter cup and spread on a paper to dry. This takes but a few minutes. Twenty grams of the dry powder is then boiled with 100 c.c. of absolute alcohol on the water-

TABLE II

TUBE	ANTIGENIC						ANTICOMPL.					HEMOLYTIC			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
A	+	++	++++	++++	++++	++++	0	0	0	+	++	++++	++++	++++	++++
A-1	++	+++	++++	++++	++++	++++	0	0	0	0	0	++++	++++	++++	++++
A-2	0	0	+	++++	++++	++++	0	0	0	0	0	++++	++++	++++	++++
A-3	0	0	+++	++++	++++	++++	0	0	0	0	+ ?	++++	++++	++++	++++
B	+	+	++++	++++	++++	++++	0	0	0	+ ?	+	++++	++++	++++	+++
B-1	++	++++	++++	++++	++++	++++	0	0	0	0	0	++++	++++	++++	++++
B-2	0	0	++	++++	++++	++++	0	0	0	0	0	++++	++++	++++	++++
B-3	0	+	+++	++++	++++	++++	0	0	0	+ ?	+ ?	++++	++++	++++	++++
X	0	0	+ ?	+++	++++	++++	0	0	0	+	++	++++	++	++++	++
Y	0	0	0	++++	++++	++++	0	0	0	+ ?	+	++++	+++	++++	+++

A = antigen prepared at low temperature, not extracted with ether or acetone.
A-1 = low temperature, extracted with ether.
A-2 = low temp. extracted with acetone.
A-3 = low temp. extracted with ether and acetone.
B = high temperature, not extracted with ether or acetone.
B-1 = high temp. extracted with ether.
B-2 = high temp. extracted with acetone.
B-3 = high temp. extracted with ether and acetone.
X and Y are control antigens prepared by the ordinary method.

under a reflux condenser for two hours. When cool, it is filtered through filter paper and the alcoholic solution containing the antigen is placed in the ice box overnight and filtered again. The antigen is now ready for titration. It gives best results in dilutions of 1 to 10 or 1 to 20

An antigen prepared according to the above described method was tested out on 500 sera, both positive and negative. It was checked against two antigens of proved value, one plain alcoholic, and one cholesterinized antigen. The following method was used: $\frac{1}{10}$ Wassermann quantities were employed in the tests. Three tubes were set up for each test: one tube containing 0.1 c.c. of diluted antigen and 0.02 c.c. of serum, another tube contained 0.05 c.c. of antigen, 0.02 c.c. of serum. The third tube contained no antigen and was used as a control. Two units of complement were added to all tubes and incubated in the ice box for four hours. Five per cent of sheep-cells and two units of amboceptor were then added and the next incubation was done in the water-bath at 37° C. for fifteen minutes

The results showed a very close agreement between the three antigens. Three hundred and thirty-nine sera gave negative results with the three antigens; 122 sera gave positive results with all the antigens; 39 sera showed a discrepancy between the three antigens. Thus there was an agreement of 92 per cent and a disagreement of 8 per cent. The disagreement was with the weakly positive and doubtful sera. However, the same disagreement existed between the two control antigens. It was never found possible to obtain identical results with different antigens in weakly positive or doubtful sera

SUMMARY

1. The beef-heart used in the preparation of antigen for the Wassermann test may be boiled or even heated in the autoclave under fifteen pounds pressure for an hour without impairing the antigen obtained by extraction with alcohol.

2. The meat may be extracted with hot alcohol without injury to the antigen.

3. A method is recommended for preparing an antigen at high temperature.

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THE CATALASE-ACTIVITY OF THE ORAL MUCOUS MEMBRANE*

By Torald Sollmann, M.D., and R. L. Howard, M.S., Cleveland, Ohio

THE susceptibility to mercurial stomatitis is generally believed to vary with the healthy or unhealthy condition of the mouth, particularly of the gums. It would be desirable if the degree of this dependence could be definitely ascertained. Such a study would be likely to be more valuable if the clinical diagnosis of the oral condition could be compared with data that would not require so much personal interpretation; and especially if these data could be reduced to a quantitative basis. It occurred to us that these requirements might be met by measuring the decomposition that hydrogen peroxide undergoes in the mouth; for oral sepsis involves the presence of pus, especially about the alveolar margin, and the production of putrefactive substances, which would probably have a reducing action.

In principle, such a method would consist of taking a quantity of hydrogen peroxide into the mouth, and estimating the amount that remained undecomposed after a certain length of time, under standardized conditions. The execution of the method is as simple as its principle and no difficulty was experienced in determining the influence of various factors of technic, and in establishing a satisfactory standardized procedure.

Through the sympathetic cooperation of Dr. W. L. Wylie, we were able to try the method in the Dental Clinic of Western Reserve University. For this purpose, a schema was worked out for observing, recording, and grading the clinical conditions from the standpoint of oral sepsis. According to this schema, the conditions of the mouth were roughly graded as "normal," "bad," and "very bad." The peroxide decomposition or "catalase index," as it may be called, was then determined. When twenty-five cases had been collected, the two values were compared. This showed that nine-elevenths of the mouths classed clinically as "normal" gave indices below a certain level; while two-thirds of the "very bad" mouths had indices materially above this level. The indices of the "bad" mouths did not exceed those of the "normal" mouths. It appears, therefore, that the catalase index reflects "very bad" clinical conditions of the mouth, but does not indicate lesions that are clinically quite evidently "bad." Even more disturbing is the fact that a few perfectly normal mouths gave a "very bad" index; and a very bad mouth gave a "normal" index. It must be recognized, of course, that there need not always be parallelism between the morphologic changes and the chemical changes that result from inflammatory processes; and the possibility of parallelism between the catalase index and the liability to mercurial stomatitis should therefore not be rejected without a trial, which has

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not yet been made. However, the results suggested that the condition of the teeth and gums is not the only, and perhaps not even the main factor to influence the catalase index of the mouth. This is confirmed by the observation that if a number of peroxide washings of the mouth are made in quick succession, the rate of decomposition remains constant, after the second washing; i.e., after the detachable oxidizable material has been removed by the first washing, the catalase action is a fixed property of the mucous membrane itself.

To throw further light on the question of the fixed catalase activity it was planned to follow the catalase index of "normal" individuals over a period of several weeks. Since their dental condition would naturally remain approximately constant during this time, the index should also remain approximately constant if it depends mainly on the dental condition. Considerable variations, on the other hand, would indicate the importance of

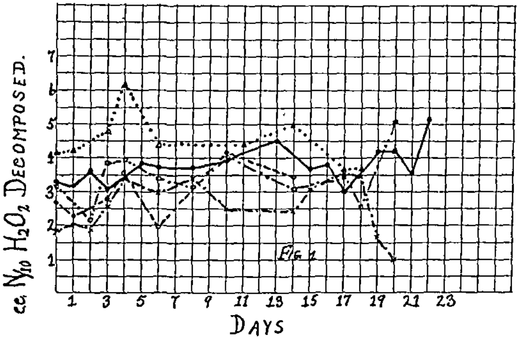

Fig. 1.—Normal course of catalase index over longer periods.

. ———— O - - - - - - - - △ . . . . X .—.—.—.—. □ . . — . . — . . — . . Indicate the curves (first peroxide treatment) for five different individuals.

nondental conditions. These tests were carried out on five persons. Fig. 1 shows that the figures for each subject ran, on the whole, fairly uniformly but marked rise or depression of the index occurred occasionally with most of the subjects. These changes may be of short or of fairly long duration.

It appears, therefore, that conditions other than dental do have a considerable influence. A few attempts were made to find such conditions; but increase of salivation by chewing paraffin, suppression of salivation by atropine, and the intercurrence of "sore throat" did not appear to have any definite effect on the index. Perhaps digestive and febrile conditions, which would modify the "coating" of the tongue, may be concerned. This constitutes a subject for future investigations. The establishment of a method for the determination of the catalase index of the mouth may perhaps also help

to solve broader questions as to the significance of the catalase activity of mucous membranes.

Method of Determining the Catalase Index of the Mouth

The following technic was fixed upon after the various factors and conditions entering into it had been investigated (as will be described in the appendix). It involves a preliminary rinsing of the mouth with 1 per cent sodium bicarbonate solution and with two portions of water, to remove mucus and detritus; then a definite quantity of titrated hydrogen peroxide solution is taken into the mouth and forced between the teeth. After one minute, the peroxide is washed from the mouth. The whole procedure is then repeated, so that two treatments with peroxide are given and two separate samples collected, and in each of these the undecomposed peroxide content is determined, at once. The first sample would contain more of any detachable material, leucocytes, etc., and may, therefore, be the better index of the presence of pus, while the second sample would reflect more definitely the catalase activity of the mucous membrane proper, and may, therefore, prove to give more important data; but until this point has been decided it appears desirable to make both determinations. In general, while the decomposition is always greater in the first than in the second sample, the two sets of figures run fairly parallel. If the samples are allowed to stand, the decomposition of the peroxide continues, and we have therefore also titrated all our specimens at the end of twenty-four hours; but the results paralleled in their general direction those of the immediate titration, except

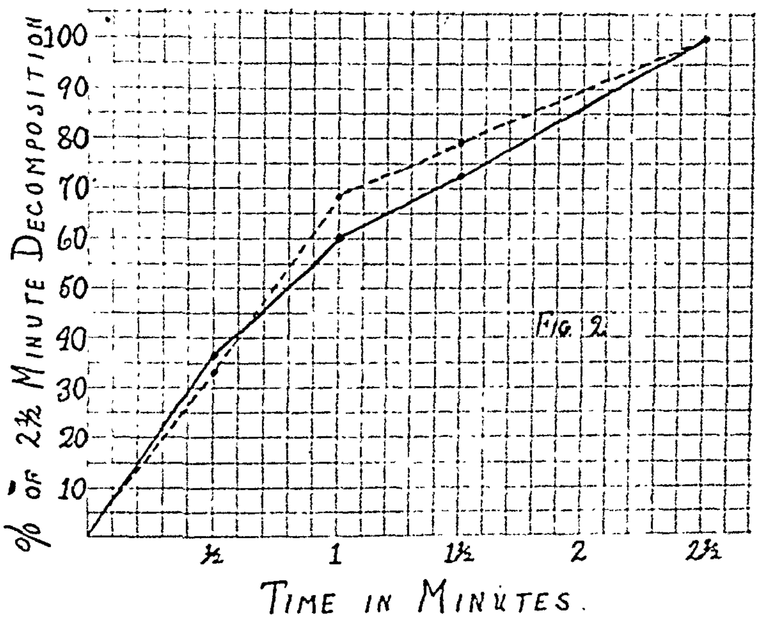

Fig. 2.—Time of sojourn of peroxide in mouth on catalase index.

———— First peroxide treatment: 100=5.40 c.c. of N/10 H_2O_2 consumed.
- - - - - - - - Second peroxide treatment in close succession after a first treatment of the same duration: 100=4.28 c.c. of N/10 H_2O_2 consumed.

that they were rather more irregular, presumably because of the slow decomposition of variable amounts of detritus. It would therefore be superfluous to reproduce our data and we would not consider the twenty-four-hour titration as worth the trouble. This reduces the details of the method to the following

STANDARDIZED TECHNIC

1. The patient is given 30 c.c. of a 1 per cent $NaHCO_3$ solution and instructed to take the entire quantity into the mouth and to rinse thoroughly both the mucosa and the teeth. The washing is discarded.

2. *Next 30 c.c. of distilled water are given to the patient, with directions* to wash mouth and teeth even more thoroughly than with the bicarbonate. This washing is discarded.

3. Another portion of distilled water is used, and No 2 is repeated.

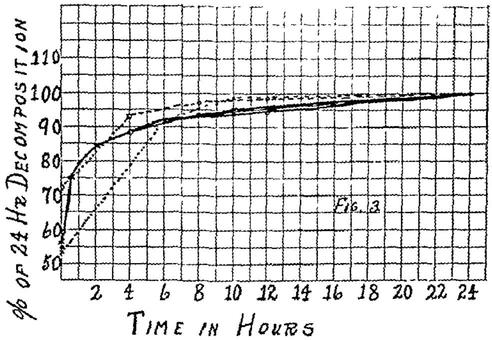

Fig. 3.—Deterioration curve of peroxide washings in vitro at room temperature.

———— First peroxide treatment (three subjects).
- - - - - - - Second peroxide treatment (two subjects).
The curve from the first peroxide treatment between zero and four-hour period is derived from a separate experiment upon one of the subjects.
The dotted lines (. . . . .) in the second peroxide treatment indicate that the form of the curve in that region has not been determined.
The absolute values for c c. N/10 peroxide decomposed in 24 and 48 hours were:

Subject	Washing	c c. Decomposed in 24 hours	c.c. Decomposed in 48 hours
12	first	3.69	3.79
H	first	3 34	3.25
H	second	1.98	2.00
11	first	3.25	3.43
11	second	1.95	2.02

4. Five c.c. of a titrated U.S.P. solution of H_2O_2 are measured carefully with a pipette and diluted with distilled water to 20 c.c. The patient is instructed to take the entire quantity into the mouth, draining the glass; then to close the teeth and force the peroxide mixture through the teeth, first front, then right side, then left side, etc., repeating this procedure for the duration of one minute. This process also washes the mucosa of the mouth. The patient is warned against getting the fluid so far back in the mouth that there will be danger of swallowing some of the peroxide; and

fore, be selected for comparative experiments. One minute was chosen because this lies beyond the steepest part of the curve and therefore beyond the region of maximal error; and on the other hand, it avoids the discomfort of too excessive foam and of the disagreeable taste that may develop if the contact is further prolonged.

Effective Contact of Peroxide with Teeth.—The standard directions for drawing the peroxide through the teeth aim to secure thorough contact. This is doubtless a wise instruction, but it need not be feared that the results will be altered if it is only negligently carried out; for comparative experiments gave practically the same results if the solution was only slightly agitated by movements of the cheeks and of the tongue.

Number of Rinsings Necessary to Obtain All the Peroxide from Mouth.—This was fixed at two, since the third washings were free from titrable quantities of peroxide.

The Catalase Index of Successive Peroxide Treatments.—When the catalase index is determined several times in sequence, on the same person, it is found that the index of the first sample is always materially higher than that of the second; but from the second sample on, the index remains constant (Table II). This shows that the catalase action is produced by the living mucous membrane. It also establishes the fact that it is not necessary to make more than two peroxide treatments to obtain the catalase index of the mucosa.

TABLE II

CATALASE INDEX OF SUCCESSIVE PEROXIDE TREATMENTS

(Cubic Centimeters of Tenth-normal H_2O_2 Decomposed After One-half Minute Contact)

SUBJECTS	A.R.	D.	W.J.C.
Peroxide Treatment 1:	2.59	2.07	1.86
Peroxide Treatment 2:	2.04	1.51	1.46
Peroxide Treatment 3:	1.90	1.49	1.23
Peroxide Treatment 4:	1.88	1.34	1.21
Peroxide Treatment 5:	1.85	1.33	1.30
Peroxide Treatment 6:	1.89	–	1.30

The Time-Curve of the Catalase Index.—This was studied by allowing the peroxide washings to stand at room temperature in a glass-stoppered bottle, and repeating the titration at intervals. Control tests on an equivalent dilution of peroxide with distilled water showed that this undergoes no change in twenty-four hours, and very little loss in forty-eight hours; after this time there is apt to be some deterioration. The results of the mouth treatments are shown in Fig. 3. In this the curves are drawn to the scale of 100 = the cubic centimeters of tenth-normal H_2O_2 decomposed in twenty-four hours, for each subject. The H_2O_2 was kept in the mouth for one-half minute. It is seen that the curves run practically parallel, for both the first and the second specimens. They have the parabolic form; so that if the decomposition at the end of twenty-four hours is taken as 100, about 58 per cent of this is decomposed within one-half minute; 85 per cent in two hours; 88 per cent in four hours; 92 per cent in eight hours; and 111 per cent in forty-eight hours.

The Daily Variations in the Catalase Index.—Repeated determinations during two or three weeks were made on five subjects with fairly normal mouths. The catalase indices were recorded only for the first washings with peroxide—one minute contact. The curves drawn from the immediate titrations are shown in Fig. 1. Their significance has already been discussed.

The Influence of Experimental Conditions—Salivation.—This was tried in two cases, by taking the one-minute contact index after chewing gum and smelling strong acetic acid. The results of two determinations are a trifle lower than the normal average of each individual, but they are well within the normal variation. This is shown in Table III.

TABLE III

THE INFLUENCE OF SALIVATION ON CATALASE INDEX

SUBJECTS:	R.L.H.	H P.C.	AVERAGE
First Sample Controls	3.76	2.96	3.36
Increased saliva flow	3.61	1.70	2.65
SUBJECTS:	R.L.H.	H.P.C.	AVERAGE
Second Sample Controls	3.12	1.48	2.30
Increased saliva flow	3.13	1.05	2.09

Suppression of Saliva by Atropine.—This was tried on three subjects each with one experiment, one minute contact. The results (Table IV) show that there is no constant effect, but only the normal variations.

TABLE IV

SUPPRESSION OF SALIVA BY ATROPINE ON THE CATALASE INDEX

SUBJECTS	R.L.H.	H.P.C.	A.R.	AVERAGE
First Sample Control	3.76	2.96	3.35	3.35
Decreased saliva flow	5.18	1.00	5.09	3.75
SUBJECTS	R.L.H.	H.P.C.	A.R.	AVERAGE
Second Sample Control	3.12	1.48	2.49	2.36
Decreased saliva flow	3.55	0.68	3.53	2.11

The Effect of Smoking.—The values shown in Table V were obtained by smoking a cigarette for ten minutes between two successive determinations; and the control experiments by permitting the same period of time to lapsé without smoking. The indices (one minute contact) run somewhat lower for the smoking, but within the normal variations, so that the difference may be accidental.

TABLE V

THE INFLUENCE OF SMOKING ON THE CATALASE INDEX

SUBJECT	R.L.H.	H.P.C.	O.W.B.	A.R.	AVERAGE
First Sample Control	4.26	3.61	3.69	3.55	3.79
Second Sample Control	3.12	1.48	2.09	2.49	2.29
First Sample (Smoking between samples)	4.26	3.55	3.71	2.67	3.54
Second Sample (Smoking between samples)	2.75	1.87	1.68	2.20	2.12

"*Sore Throat.*"—One of the subjects, who was under observation for three weeks, to determine the variability of the normal index, developed a

cold and "sore throat," sufficiently severe to confine him to bed; the catalase index, however, ran a practically steady, normal course; so that in this case, at least, the pharyngitis did not affect the catalase index.

The Range of the Catalase Index in Normal and Pathologic Conditions of the Mouth.—The data for this study were secured from the Dental Clinic of Western Reserve University through the kind collaboration of W. L. Wylie.

A careful clinical examination was made before the chemical tests; recording especially such conditions as might indicate oral sepsis: The odor of the breath (which was found a very useful criterion); caries; the condition of the gums, especially recessions and pyorrhea; the condition of the mucosa of the various portions of the mouth; and the presence of any definite lesions, patches or ulcers. According to this examination, the patients were grouped as

N = *Normal,* i.e., without definite inflammatory conditions;

B = *Bad,* with definite active caries, receded, or inflamed gums, or patches; and

BB = *Very bad,* with marked inflammatory conditions, and especially pyorrhea or other distinctly pathologic lesions.

The indices of the first and second tests are shown in Table VI, arranged in the order of the magnitude of the index of the first test. The fourth column gives the clinical grouping.

TABLE VI

CATALASE INDEX (1 MINUTE CONTACT) OF NORMAL AND PATHOLOGIC MOUTHS

CASE NUMBER	FIRST TEST INDEX CATALASE	SECOND TEST INDEX CATALASE	OF MOUTH* CONDITION CLINICAL
20	1.35	1.35	B
25	1.36	–	B
16	1.81	1.15	N
22	1.82	1.27	N
26	2.04	–	B
6	2.15	–	N
24	2.35	–	B
30	2.50	0.61	BB
5	2.96	–	N
2	2.96	–	N
3	3.35	–	N
18	3.39	2.11	N
21	3.41	2.27	N
23	3.44	–	B
14	3.67	2.14	BB
1a	3.76	2.44	N
29	3.88	2.19	B
17	4.16	1.74	B
4	4.33	–	N
27	4.53	4.88	B
19	5.51	4.60	N
15	5.88	5.50	BB
13	5.95	5.05	BB
31	6.69	5.04	BB
28	8.20	4.71	BB

*N—"Normal."
B—"Bad."
BB—"Very bad."

Table VII gives the range medians for the clinical groupings.

TABLE VII

THE CATALASE INDEX OF THE CLINICAL GROUPS

GROUP	CATALASE INDEX OF FIRST TEST:			CATALASE INDEX OF SECOND TEST:		
	No. of Cases:	Range:	Median:	No of Cases:	Range:	Median:
Normal	11	1.81-5.51	3.23	7	1.15-4.60	2 27
Bad	8	1.35-4.53	3.44	4	1.35-4.88	1.96
N plus B	19	1.35-5.51	3.33	11	1.15-4.88	2.19
Very bad	6	2.50-8.20	5.95	6	0.64-5.50	4.87

It is evident that the "normal" and "bad" cases must be grouped together as "normal," so far as the catalase index is concerned. A fairly definite dividing line between these and the "very bad" cases lies at 4.0 for the first washing and 3.0 for the second washing; of the "normal to bad" cases, three-fourths (i.e., fifteen-nineteenths) lie below 4.0 for the first washing and nine-elevenths lie below 3 0 for the second washing; whereas of the "very bad" cases, two-thirds (i.e, four-sixths) lie above this dividing line, both for the first and for the second washing.

THE POTENTIATION OF NOVOCAINE SOLUTIONS*

BY WILLIAM R. MEEKER, M.D., MOBILE, ALA.

IN the production of novocaine the efforts of workers in the field of synthetic drugs have been rewarded with decided success. For several years this drug has most nearly fulfilled all the requirements of the ideal local anesthetic, almost entirely superseding other substances. While conceded to be somewhat less powerful in anesthetic action than cocaine, it has gained a reputation for efficiency which, when considered with its high solubility, stability in solution even when sterilized by boiling, its low toxicity and freedom from local irritating effects, has placed it at the top of the list in point of general applicability. One of the most important advantages of novocaine is the possibility of safely injecting large quantities of the solutions. This fact has greatly broadened the scope of local anesthesia and has been largely responsible for the development of regional anesthesia upon its present scientific basis.

While novocaine is the least toxic of local anesthetic substances, it must be remembered that all such drugs are protoplasmic poisons with greater or less affinity for nerve cells. The toxicity of novocaine is not an entirely negligible factor. When local infiltration was limited to minor operations the injection of small quantities of weak solutions kept the method well within

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the limits of safety. But with the introduction of regional and nerve block methods the total quantity and concentrations of the solutions have increased. In certain quarters deaths have been reported and the local anesthetic held responsible. These deaths have not only been those in which large amounts of solutions have been employed. Fatalities have followed the injection of small amounts of solution in cervical plexus block, and in sacral anesthesia.[24] Of a total of forty-three deaths investigated by a special committee of the American Medical Association,[14] twenty occurred during the performance of tonsillectomy. It is gratifying to observe, however, that of the total number of deaths novocaine was employed in only two cases, although it enjoyed a far wider clinical use than any other drug.

While the safety of major operations rendered anesthetic by the use of large quantities of novocaine solutions properly employed has been repeatedly demonstrated, the sphere of usefulness of the method would still be farther extended by the introduction of a drug even less toxic than novocaine. It is reasonable to expect that synthetic chemists will produce a local anesthetic drug having a greater margin of safety than novocaine. Many such intended preparations have been the subject of pharmacologic investigation. At the present time study of upwards of thirty new preparations is being carried out under McGuigan's[15] directions at the University of Illinois. Until the superiority of some new preparation has been demonstrated, however, the only means of improvement consists in the possible potentiation and prolongation of the anesthetic action of those drugs now in use.

Various methods have been proposed for the potentiation of novocaine solutions. For all of these it is claimed either that the anesthetic power of the novocaine is increased to such an extent that satisfactory anesthesia may be obtained with weaker solutions, or that the duration of the period of anesthesia is prolonged. The only method of universally acknowledged benefit has been the addition of adrenalin. To Braun[2] is largely due the credit of first introducing and perfecting the use of this agent in local anesthesia. There is probably no other single factor which has more encouraged the use of local anesthesia. By means of the vasoconstriction of adrenalin the anesthetic solution is retained within the tissues longer, which both intensifies and prolongs the effect for a period of time usually sufficient for the performance of most major operations.

Sodium bicarbonate has been used as an adjuvant to novocaine. Gros[6] determined experimentally that the bases of local anesthetics have a more intense action than their salts for the reason that they act more quickly and in weaker solutions than their salts. At first he added the amount of sodium bicarbonate calculated to bind the chloride, later finding that an excess of alkali gave still greater efficiency so that isotonic solutions of sodium bicarbonate or phosphate were used. He maintains that a novocaine-sodium bicarbonate solution is five times as active as an equimolar novocaine hydrochloride solution. This is explained by the liberation of the free bases which penetrate the nervous tissues more readily than the water soluble salts. The anesthetic potential of novocaine salts was thus thought to depend upon the degree to which the free base was dissociated. Läwen[12] made use of this

principle by adding sodium bicarbonate to his formula for sacral anesthesia, which is still in general use in Germany. Sollman[22] confirmed Gros's results in experiments upon frogs and rabbits but in applying it to the human skin he found very disappointing results, the alkaline solutions being less anesthetic than the same solutions without the alkali. Gros and Läwen describe favorable effects both experimentally and clinically. On the other hand Allen[1] and Labot[12] caution against the danger of adding alkali to the anesthetic solution. They even advise against sterilizing of the instruments in an alkaline solution as being a source of possible contamination which is destructive to the anesthetic agent. Others maintain that the alkali splits the adrenalin. *Braun found the addition of alkali to be inferior in duration, intensity* and diffusive power to the ordinary method of preparation which gives a solution nearly neutral in reaction. Meeker and Scholl[16] were unable to observe any benefit in the addition of sodium bicarbonate to the formula for sacral anesthesia.

Potassium sulphate was first used as a means of potentiation by Hoffmann and Kochmann.[10] Their experimental work included both the sciatic nerve of the frog and the intracutaneous test on man. By the former method a potentiation of two and two-thirds times was observed and by the latter *five times in terms of dilution.* They recommend *the addition of 0.4 to 2* per cent potassium sulphate to the novocaine solution. As the result of experimental work Sollman concluded that mixtures of the anesthetic with potassium sulphate give only simple summation and not potentiation in terminal infiltration anesthesia. After a wide clinical experience Farr[4] has been unable to satisfy himself that with the exception of adrenalin any of the drugs recommended for potentiation possess any considerable advantage.

Calcium chloride has been employed to improve the anesthetic solution. Harris[8] holds that this chemical greatly prolongs the anesthesia probably by delayed absorption of the solution. *He adds from 0.25 to 1 per cent depending* upon the length of time it is desired the anesthesia should last. He also recommends the addition of both calcium chloride and potassium sulphate. In spite of these claims for the efficiency of calcium chloride it has never found favor with other surgeons.

Sodium sulphate was added by Strauss[23] to improve his formula for sacral anesthesia. The advantage he claimed was that it prevented the decomposition of adrenalin, thereby prolonging anesthetic action.

Chloretone has also been used as an adjuvant. Harris[8] adds 1 per cent chloretone (chlorbutanol) as sterile crystals *after the solution has cooled.* It is soluble only to the extent of eight parts to a thousand, so that the addition of 1 per cent simply insures a saturated solution, and the remaining few undissolved crystals settle at the bottom as a slight harmless deposit. Chloretone is itself a powerful local anesthetic also a hypnotic and antiseptic agent. Injected hypodermically into the tissues it is quite irritating but is followed by marked anesthesia, the site of injection remaining later as a painful induration for some time. According to Harris it intensifies the action of novocaine although it seems never to have been employed by other surgeons for this purpose.

Quinine urea hydrochloride and novocaine do not synergize. But it has been suggested that the addition of quinine urea hydrochloride to the novocaine solution may prolong the anesthesia. The long duration of the anesthesia resulting from the injection of quinine salts alone in strengths of 1 per cent and greater is due to an exudate of fibrin.[9] Anesthesia may last from several hours to four or five days. It is questionable whether the prolonged anesthesia is sufficient compensation for the risk of delayed union and the possibility of an occasional slough. But the addition of a small amount of the drug, 0.1 to 0.2 per cent, to the novocaine solution to prolong the anesthesia might prove a decided advantage, as for example in block of the sacral nerves in obstetrics.

Oils and Gelatine.—Prolongation of anesthetic action has been attempted by dissolving the novocaine in a medium of oil. This has been found upon practical tests to be unsatisfactory and to possess many disadvantages. Oils are absorbed only by the lymphatics which act more slowly. This prolonged retention in the tissues should prolong the local effect and permit it to be almost entirely exhausted locally. But such solutions are entirely impractical due to the poor diffusion of oily substances through the tissues and consequent weak anesthetic action. They may also act as local irritants.

Muroya[16] has shown in experiments upon rabbits that 5 per cent gelatin in the solution delays its absorption and increases its efficiency. Allen employed gelatine in sacral injections but was uncertain of any advantages. Braun has failed to notice any increase in local effect from cocaine by the addition of gelatine. He sterilized the solutions carefully which according to Klapp[11] so alters the gelatine that its property of delaying absorption is lost.

Magnesium salts have been given considerable prominence through the writings of Meltzer.[17] The intracerebral injection of magnesium sulphate induces a state of general inhibition. Subcutaneous and intravenous injections produce deep narcosis and complete muscular relaxation. The application of magnesium sulphate to nerve trunks has been shown to block conductivity and abolish excitability. Spinal anesthesia has been produced and painless operations performed below the site of injection. This salt has also been employed as an adjuvant in general narcosis and for its alleged synergistic action with morphine. No record is available of its effect when added to a local anesthetic solution so that the possibility of potentiation remains to be investigated.

METHODS

The comparative anesthetic activity and toxicity of local anesthetic drugs has been investigated experimentally ever since substitutes were proposed for cocaine. The possibility of potentiation of these drugs has received but very little experimental investigation. The large mass of conflicting clinical data which has accumulated is of little help in understanding the value of the various mixtures. In view of the great practical importance of the subject, it has seemed worth while to carry out the following investigation. The current experimental methods of determining anesthetic potential have

been followed closely in determining the values of the potentiation formulas. Among laboratory tests that have been devised for this purpose there are six, each of which appears to have a bearing upon the clinical application; (1) Block of the sciatic nerve of the frog by direct application to the nerve trunk; (2) Paralysis of sensory nerve fibers of the frog's sciatic by direct application of the lumbosacral plexus; (3) Surface anesthesia of the frog's skin by immersion; (4) Anesthesia of the rabbit's cornea by irrigation of the conjunctival sac; (5) Mucous membrane anesthesia of the human tongue by immersion; (6) Cutaneous anesthesia in the dog and man by dermal wheals produced by intracutaneous infiltration.

I. BLOCK OF THE SCIATIC NERVE OF THE FROG

Paralysis of the motor fibers by direct application to the sciatic nerve of the frog is a method very commonly used for testing local anesthetic action. It is simple in application and outside factors modifying anesthetic effect are reduced to a minimum. For this reason it would appear that results should be more exact.

Method.—The neuromuscular preparations were made so as to include the lower end of the leg from the knee down, and the entire sciatic nerve from the knee to where it enters the pelvic girdle. The nerve was carefully isolated by blunt scissors dissection without traction or other trauma to the nerve trunk. Preparations were used as soon as made, always being tested beforehand and found active.

In applying the test the method described by Sollman was followed. The nerve trunk was immersed in the solution contained in a small trough cut into a block of paraffin. The excitability was tested with the platinum electrode of an induction coil, the usual apparatus for stimulation in laboratory experimentation. The same strength of current was used for stimulation of all preparations, and was the least that could be detected when the electrodes were placed on the tip of the tongue. The stimuli were applied one centimeter from the cut end of the nerve. Paralysis seems always to travel from

TABLE I

TIME REQUIRED FOR BLOCK AND RECOVERY OF THE SCIATIC NERVE*

SUBSTANCES	8%	4%	2%	1%	½%	¼%	⅛%	1/10%	
Magnesium sulphate	0	0	0						
Gelatine		0	0	0					
Sodium sulphate	50+30	0							
Calcium chloride	58+34	0	0						
Sodium bicarbonate	45+36	0	0						
Sodium chloride	33+34	0	0						
Chloretone				[illegible]	[illegible]	[illegible]		0	
Potassium sulphate	11+21	13+21						0	
Potassium chloride	7+14	16+13						0	
Potassium bicarbonate	[illegible]	[illegible]						0	
Quinine urea HCl.									
Novocaine								0	
Cocaine								0	
Distilled water									40+32
Desiccation									16+4

*The number to the left of the + indicates the number of minutes required for the production of nerve block. The number to the right of the + denotes the minutes required for recovery. The same system is followed in other tables.

the cut end of the nerve toward the muscle so that when the block was complete in the distal segment of the nerve a response could still be elicited by moving the electrodes toward the muscle. This probably indicates difficulty in penetration of the nerve sheath proper, and more rapid diffusion from the cut end of the nerve trunk between fasciculi. The same feature was observed, however, when the cut end was tied with fine thread and when only an intermediate segment of the nerve trunk was immersed.

Preparations were tested at five-minute intervals by grasping the tip of the nerve trunk with a fine iris forceps, lifting it out of the trough and applying the electrode at a point a centimeter from the end. The complex and troublesome pieces of apparatus described by Läwen and Clossen[3] for stimulation without handling the nerve trunk are unnecessary, especially when one considers the great limitations of the method itself, as will later be pointed out.

The end point was considered reached when no muscular contraction whatever could be elicited. Irritability does not cease suddenly and the criteria of paralysis have probably been applied differently by different investigators. After complete paralysis the preparations were kept in physiologic salt solution and tested at five- to ten-minute intervals for the return of function.

All experiments were conducted at room temperature. The ordinary Leopard frog (Rana pipens) was used. Substances to be tested were dissolved in physiologic salt solution (0.9 NaCl.) unless otherwise stated. The concentration of the drugs was in arithmetical progression, 8, 4, 2, 1, ½, ¼, ⅛, 1/16, and 1/32 per cent respectively.

Results.—In order to distinguish simple summation of anesthetic actions from true potentiation it is first necessary to determine the action of each constituent of the mixtures separately. For all practical purposes if complete block does not occur within an hour it had best be disregarded. Table I expresses the comparative actions of these substances. Each result represents the average of several trials. Cocaine has also been included for comparison. It is shown that cocaine, novocaine and potassium salts are approximately equally anesthetic. The small variations might well be within the limits of experimental error. Chloretone in saturated solution (0.8 per cent) and ½ per cent strength is twice as powerful as cocaine and novocaine, while quinine urea hydrochloride is inferior. The action of the inert salts in 8 per cent concentration is not to be regarded as an anesthetic action, but more the effect of any highly concentrated salt solution with no anesthetic power, although C. P. sodium chloride blocked in thirty-three minutes while magnesium sulphate was ineffective after an hour. A hypotonic effect is seen in the action of distilled water which blocked within forty minutes. Desiccation, of course, produces prompt nerve block which must be guarded against especially in the small segment of the nerve crossing from the paraffin trough to the muscle.

The potentiation values of the mixtures is best measured by mixing equal parts of novocaine and the solution to be tested for potentiation. Minimal effective doses as used by Sollman are very difficult to determine and

must be correspondingly inaccurate. The velocity index, or speed of paralysis, is more accurate than the index of dilution or minimal effective concentration in addition to being an easier and simpler method.

Table II expresses the results of the potentiation tests. Calcium chloride, magnesium sulphate, sodium sulphate and gelatine were all chemically inert alone and likewise showed no potentiation whatever. These values are approximately the same as those of novocaine alone. Mixtures of quinine urea hydrochloride are less active than the same strength of novocaine alone. Investigators have consistently shown that there is no gain in anesthetic power by the addition of other local anesthetic drugs to the novocaine solution. The novocaine-chloretone values represent simple summation without potentiation: that is, the combined action of the ¼ per cent strengths block in the average of their half per cent strengths acting alone. When potentiation has taken place the velocity of action should be shorter than the average of

TABLE II

ACTIVITY OF POTENTIATION MIXTURES

MIXTURES	½%	¼%	⅛%	1/16%	1/32%	
Novocaine and calcium chloride	35+25					
Novocaine and magnesium sulphate	36+12					
Novocaine and sodium sulphate	34+22					
Novocaine and gelatine	39+18					
Novocaine and quinine urea HCl.	46+21					
Novocaine and chloretone		18+15	37+21	0		
Novocaine and potassium sulphate	13+21	27+19	50+21	0		
Novocaine and potassium chloride	12+20	29+18	34+20	40+24	0	
Novocaine and potassium bicarbonate	5+18	9+11	14+13	30+23	63+32	0
Novocaine and sodium bicarbonate	7+21	13+18	16+14	30+22	49+15	0

TABLE III

ANESTHETIC ACTIVITY OF NOVOCAINE AND HALF PER CENT ALKALI IN DISTILLED WATER

NOVOCAINE	½%	¼%	⅛%	1/16%	1/32%	1/64%	1/128%	1/256%
Sodium bicarbonate ½%	7+13	9+20	10+20	14+16	22+15	37+20	50+27	0
Potassium bicarbonate ½%	5+21	6+18	8+12	11+12	19+17	15+17	23+27	39+22

TABLE IV

COMPARATIVE ACTIVITY OF NOVOCAINE AND HALF PER CENT SODIUM BICARBONATE IN DISTILLED WATER AND IN PHYSIOLOGIC (0.9 PER CENT) SALT SOLUTION

½ SODIUM BICARBONATE AND NOVOCAINE	½%	¼%	⅛%	1/16%	1/32%	1/64%	1/128%
Physiologic salt solution	7+21	13+18	16+14	30+22	40+15	0	
Distilled water	7+13	9+20	10+20	14+16	22+15	37+20	50+27

the next higher dilution. Novocaine and potassium sulphate have a combined action in ½ per cent solution of 13 + 21, which approximates the velocity of the 4 per cent strengths working alone. The average of the next higher dilution of each acting separately (37 + 35 and 27 + 23) is 32 + 29. The combined action of the two therefore gives a velocity index higher than the average of the sum of their separate actions, which is an example of true synergism or potentiation. The extent of potentiation is roughly two or three times. A similar result is seen in the combined action of novocaine and potassium chloride. The action in combination with the alkalis was still

further intensified, approximately four or five times. These results compare favorably with those of Gros and Sollman. Gros at first added just sufficient alkali to bind the hydrochloride. He found later that an excess of the bichloride was still more efficient and then employed isotonic bicarbonate solution. We tested this principle by combining higher dilutions of novocaine with a constant concentration of alkali (½ per cent). Since the constant content of the bicarbonate remained nearly isotonic, distilled water was employed as the solvent instead of physiologic salt solution. These results expressed in Table III are the highest values obtained. Of the two alkalies potassium is the more preferable when a constant concentration is to be employed, because of the anesthetic action of the potassium ion as well as the potentiation of the alkali. When equal mixtures of alkali and novocaine are employed sodium seems to be the equal of potassium.

An attempt was made to determine the influence of the solvent by comparing mixtures of novocaine and sodium bicarbonate in distilled water with the same mixtures in physiologic salt solution. These results expressed in Table IV indicate slightly better action with distilled water as the solvent. This difference might be within the limits of experimental error, but it is quite constant in all dilutions and all values represent the averages of several trials.

Discussion.—This method has been criticized because it is a determination of motor nerve block and therefore does not measure the efficiency on sensory fibers. It should be of comparative value, however, for the velocity of block of motor nerves is a fair indication of comparative action on sensory nerves, the latter being affected more easily. The results cannot be transferred to clinical practice since the conditions of absorption are not reproduced. On the other hand the accuracy of measurement is not influenced by many factors which are present in the living, such as the presence of the solution within the tissues, its tonicity and diffusibility and the site and mode of injection.

The data of different investigators using this method have varied widely. Absence of response to stimulation depends, as shown in these experiments upon the solvent, the strength of the stimulating current and especially upon the distance of the point stimulated from the end of the nerve. Sollman mentions other factors such as the length of the immersed segment, temperature and size of the nerve trunk. The writer is unable to state whether these factors increase or decrease the velocity of block. Of far greater importance is the individual variation of different frogs. This feature is easily sufficient to account for discrepancies of different investigators. The normal survival time of twenty preparations placed in pans of salt solution and covered with filter paper varied between four and sixteen hours or 400 per cent. Differences in velocity of block of 100 per cent are occasionally seen, and it is not rare to observe the half per cent solution becoming effective before the one per cent. Averages of several trials must therefore be taken, since the factors governing individual variation are unknown. This feature makes the method one of coarse approximation only, and inherently incapable of the refined accuracy usually associated with a scientific quantitative method.

II. BLOCK OF THE SENSORY FIBERS IN THE SCIATIC NERVE OF THE FROG

The efficiency of local anesthetics on sensory fibers of animals is difficult to estimate. The sciatic or brachial nerve of rabbits has been isolated, sprinkled with the anesthetic drug and the time of paralysis observed. The same method has been followed in the determination of the concentration required to produce complete anesthesia, but these methods are not practical for the present study. The method of Sollman of applying the solution to the lumbosacral plexus of the frog is of much greater quantitative value.

Method.—The frogs were decapitated leaving the lower jaw then eviscerated through an incision across the abdomen just below the sternum. The lower abdominal cavity thus forming a little pocket was then filled with small cotton pledgets saturated with anesthetic solution, so that the lumbosacral trunks throughout their course within the abdomen were bathed in the solution. At intervals of from five to ten minutes the persistence of the reflex path was tested by immersing the feet in N/10 hydrochloric acid. According to Sollman disappearance of the reflex indicates destruction of the reflex arc by interruption of conductivity in the sensory fibers. He states that after thirty or forty minutes the paralysis may be due to death of the reflex centers and should not be counted.

At the time of complete disappearance of the reflex the excitability of the motor fibers was tested by stimulation of the plexus. This was successful in every case and remained active for from ten to thirty-five minutes after paralysis of the sensory fibers, the plexus being bathed all the while in

TABLE V

MINUTES REQUIRED FOR COMPLETE BLOCK OF SENSORY FIBERS OF THE FROG'S SCIATIC

	2%	1%	½%	
Physiologic salt solution				80
Tap water				72
Distilled water				52
Quinine urea HCl.	40	84	0	
Novocaine	7	19	29	
Potassium bicarbonate	6	12	37	
Potassium sulphate	5	15	22	
Magnesium sulphate		85		
Sodium chloride		75		
Calcium chloride		70		
Sodium bicarbonate		70		
Gelatine	85			
Sodium sulphate		69		
Chloretone			77	

TABLE VI

ANESTHETIC POWER OF POTENTIATION MIXTURES ON SENSORY FIBERS OF FROG'S SCIATIC

	½% EACH	¼% EACH	⅛% EACH	1/16% EACH
Novocaine and magnesium sulphate	30			
Novocaine and calcium chloride	27			
Novocaine and sodium sulphate	32			
Novocaine and quinine urea hydrochloride	40			
Novocaine and gelatine	35			
Novocaine and potassium sulphate	9	23	45	
Novocaine and potassium bicarbonate	8	15	40	64
Novocaine and sodium bicarbonate	9	14	35	60

anesthetic solution. This action is comparable to the earlier effect upon sensory than motor fibers sometimes seen in a light spinal anesthetic in which a leg is painlessly amputated without motor paralysis, the patient being able to move or raise it himself.

Results.—The survival period of such preparations kept in salt solution was from one and a half to two and a half hours. Contrary to Sollman's findings, when the reflexes had once disappeared they did not return, so that the period of recovery could not be estimated. Preparations were kept in saline for from two to five hours testing for recovery, still no restoration of reflexes was seen. The results expressed in Table V, therefore, indicate velocity of anesthetic action only. Novocaine and potassium salts are equally active. Quinine urea hydrochloride is much less powerful, while chloretone may be classed with the inert salts.

No potentiation was observed by this method except with potassium salts and alkalis. As in paralysis of motor fibers, the potentiation with potassium was less than that of the alkalis (Table VI). Potentiation is stronger when an excess of alkali is used, that of potassium bicarbonate being better than sodium bicarbonate, especially in the higher dilutions (Table VII).

TABLE VII

ANESTHETIC POWER OF NOVOCAINE WITH EXCESS OF ALKALI

NOVOCAINE	½%	¼%	⅛%	1/16%	1/32%	1/64%	1/128%
½% Sodium bicarbonate	7	13	30	45	62	77	0
½% Potassium bicarbonate	8	13	16	19	24	24	30

Discussion.—The efficiency of local anesthetics on sensory fibers is difficult to estimate because of the introduction of the complicating factor of the reflex arc. It is aided in this case by removal of the higher nerve centers and destruction of circulation. Paralysis would occur as well with interruption of the reflex pathway at other points than along the afferent fibers, as for example, at the reflex center. This could occur from absorption on the anesthetic drug. Considerable surface is bathed within the frog's abdominal cavity which, with diffusion and lymphatic absorption and the action of lymph hearts might produce a systemic action sufficient to inhibit the reflex centers. This feature is offered as a possible explanation of the failure of reflexes to return.

As a method of quantitative determination of anesthetic action it is unreliable and can only give crude approximations. It is certainly not sufficiently sensitive or constant to be of much clinical value alone and must be considered together with other methods.

III. SURFACE ANESTHESIA OF THE FROG'S SKIN

Application of anesthesia solutions to the skin of the frog is a method more suited to the most powerful anesthetics. Novocaine is of little clinical value as a surface anesthetic, its only use being in the urethra and its value there is questionable. It is not as well suited for anesthesia of mucous membranes as cocaine and a few other substances which penetrate this structure more readily. This method was tried along with the others in the

hope that the potentiation formulas might prove as powerfully anesthetic as cocaine.

Method.—Frogs were decapitated leaving the lower jaw. The viscera were removed and the body cavity washed out. The right leg was then immersed as high as the knee in a test tube of anesthetic solution. At the end of ten-minute intervals both legs were tested by immersion in N/10 HCl about half way to the knees. When both legs were simultaneously withdrawn the substance was ineffective; if the right leg was not withdrawn sensation was absent. The test was then performed on the left leg without control.

Results.—Table VIII shows the comparative activity of the different substances acting alone. Preparations remain active for periods of from one to two hours. The inert salts evidently possess slight anesthetic qualities when sufficiently hypertonic. Potassium salts and novocaine remain about the same, while quinine urea hydrochloride is inferior, as seen in the other tests. The surprising feature is the high efficiency of chloretone in half per cent solution, which is approximately the equal of cocaine in 2 per cent solution. This value for cocaine is much lower than that given by other investigators. The values for individual trials with 2 per cent cocaine varied between five and twenty-five minutes. One value of thirty-five minutes was thrown out. The average value of twelve minutes is near Sollman's value for half per cent novocaine. Recovery periods are not included since recovery is very rarely observed even when washed in tap water.

TABLE VIII

COMPARATIVE ACTIVITY OF SUBSTANCES ACTING ALONE

	4%	2%	
Tap water			105
Physiologic saline			75
Distilled water			85
Gelatine		70	
Sodium chloride	27	31	
Sodium bicarbonate	40	54	
Magnesium sulphate	31	50	
Calcium chloride	50	41	
Sodium sulphate	40	45	
Potassium sulphate	19	45	
Potassium chloride	18	30	
Potassium bicarbonate	20	40	
Quinine urea hydrochloride	35	40	
Chloretone, ½ and ¼%	11	19	
Novocaine	20	45	
Cocaine		12	

Table IX expresses the activity of the potentiation mixtures. The inert salts are of no value. Chloretone and novocaine give simple summation. The same potentiation with alkalis is seen as in the previous methods. Novocaine plus alkali is the equal, if not the peer, of the same strength of cocaine.

Discussion.—Braun criticizes results by this method as applying only to the skin of the frog, the physiologic properties of which must materially influence the local action of the substance. He failed in many instances to observe any stimulation with many very irritating solutions and maintains that a comparison of these results with other methods is not possible as long

TABLE IX

ACTION OF POTENTIATION MIXTURES

NOVOCAINE	ONE PER CENT
1% Magnesium sulphate	30
1% Sodium chloride	30
1% Calcium chloride	28
1% Sodium sulphate	35
1% Potassium chloride	27
1% Potassium sulphate	50
2% Gelatine	45
1% Potassium bicarbonate	17
1% Sodium bicorbonate	13
1% Quinine urea hydrochloride	33

as the permeability of frog's skin for various substances is unknown. We find roughly, with the exception of chloretone, the same ratio of anesthetic action by this method as in I and II and cannot criticize it on that score. Our objection to the method is in the introduction of the complicating factor of the reflex arc and the marked individual variation. Interruption in conductivity of the reflex arc may take place at other points than at the sensory terminals. Sollman states that after forty minutes the results become unreliable because late reactions depend upon the survival period of the reflex centers. This feature would also account for a failure of the reflexes to return. Individual variation of from one to five hundred per cent is often seen. It is this feature which accounts for the marked discrepancies among the different workers. Hamilton[7] states that this method represents a reaction capable of accurate measurement, and as such should take its place as a valuable pharmacologic assay method for establishing relative values. It is true that the reaction can be accurately measured; but when the absence of reaction may depend upon other things than paralysis of sensory terminals and when individual differences due to unknown factors are so marked, results will continue to be quite variable. Gradenwitz[5] observed even finer distinctions with this method than Hamilton. If the leg to be tested was drawn out of the acid before the control leg he assumed that the substance had increased sensibility; if the control leg was drawn out sooner than the leg to be tested it represented a decrease in sensibility. In our experience this method is incapable of such a refined degree of quantitative distinction.

IV. ANESTHESIA OF THE RABBIT'S CORNEA BY IRRIGATION OF THE CONJUNCTIVAL SAC

A common method of testing surface anesthesia is the irrigation of the conjunctival sac. This test involves both penetration of the mucous membrane and action upon nerve terminals. Sollman states that its results can be transferred directly to clinical practice.

Method.—The rabbits were wrapped snugly in a towel or apron leaving the head free. The winking reflexes were tested by touching the cornea with the tip of a lead pencil. The lashes were clipped closely. The lower lid was pinched into a pocket which was filled with solution by means of an eye dropper. The conjunctiva was thus bathed for *one minute.* At five-minute intervals the winking reflex was tested by touching the cornea with the

point of a lead pencil over a period of twenty minutes. The end point was regarded reached when the pencil could be drawn across the cornea with moderate pressure without causing winking. Rabbits in which the test was positive were not used again on the same day thus allowing ample time for recovery. This is essentially the method described by Sollman.

Results.—The results of individual action of these substances are shown in Table X. The inert salts, potassium salts, chloretone, and quinine urea hydrochloride were all inactive. Novocaine was only questionably positive on two trials and entirely without effect in four. Cocaine anesthetizes absolutely and promptly in 2 per cent solution.

TABLE X

COMPARATIVE ACTION UPON THE CONJUNCTIVA OF THE RABBIT

	4%	2%	½%
Magnesium sulphate	0		
Calcium chloride	0		
Sodium chloride	0		
Sodium sulphate	0		
Sodium bicarbonate	0		
Potassium chloride	0		
Potassium bicarbonate	0		
Potassium sulphate	0		
Quinine urea hydrochloride	0		
Chloretone			0
Novocaine	0		
Cocaine		2 + 30	

TABLE XI

ACTIVITY OF POTENTIATION MIXTURES

NOVOCAINE	2 PER CENT
2% Magnesium sulphate	0
2% Sodium sulphate	0
2% Calcium chloride	0
2% Potassium sulphate	0
2% Potassium chloride	0
¼% Chloretone	0
2% Quinine urea hydrochloride	0
2% Potassium bicarbonate	5 + 25
2% Sodium bicarbonate	5 + 25

The potentiation mixtures, Table XI, showed no activity in two per cent mixtures with potassium salts. Corneal anesthesia was observed only when novocaine was combined with alkali. This action must be regarded as potentiation since both substances were inactive alone in 4 per cent solution. This constitutes further proof that the addition of the alkali to the novocaine solution increases the power of penetration as well as the anesthetic action.

Discussion.—The attitude of investigators toward the efficiency of this method varies. Sollman is an enthusiastic supporter of the method while Closson finds it unsatisfactory. Our first criticism of its value is the great individual difference in rabbits. Rabbits of a sluggish temperament often tolerate touching of the cornea with the pencil point when no anesthesia is present. We found rabbits which we discarded as unsuitable for the test because of this factor. Rabbits of the opposite temperament, those which resist handling and are alert and excited often wink before the pencil has

touched the cornea even with the cocaine anesthesia. The chloretone solution may have been anesthetic, but it is apparently so irritating as to compromise the determination of an end point. The criteria of the end point are also purely arbitrary and have been applied differently by different investigators. The end point will vary with the amount of pressure employed in drawing the pencil across the cornea, the number of repetitions and whether the point of the pencil is smooth or presents fine gritty material which will scratch the cornea. Winking often occurs sluggishly after repeated stimulation with marked pressure. In such instances one is uncertain whether there is partial anesthesia or absence of anesthesia in an animal of naturally sluggish reactions. As an accurate quantitative test the ophthalmic method is unsatisfactory and suitable only for rough approximations. Even then, results of different investigators will not be comparable and the same ratio of anesthetic activity by the different methods of laboratory investigation may not be constant. For this reason no attempt has been made to compare the efficiency of novocaine plus bicarbonate with cocaine employing a series of dilutions of each.

V. MUCOUS MEMBRANE ANESTHESIA OF THE HUMAN TONGUE BY IMMERSION

Anesthesia of the buccal mucous membranes was one of the earliest local anesthetic effects to be observed. The sensation of numbness resulting from chewing coca leaves suggested its use as a surface anesthetic. Surface anesthesia of unknown solutions may be demonstrated by applying them to the tongue or gums. The anesthetic effect is manifested by a sensation of numbness very easily recognized. It is an attractive method because it is essentially the same as that of clinical practice.

Method.—The tip of the tongue was immersed in the solution for two minutes, after which the mouth was washed out and the tongue tip tested for anesthesia by rubbing it against the cutting margins of the incisor teeth. An attempt was also made to test anesthesia by pricking the tongue tip with a needle but this was unsatisfactory. The needle prick may be painful even with mucous membrane anesthesia. All tests were made upon the writer's tongue. Tests were performed in succession with the inert substances, but when anesthesia resulted it was necessary to wait until there had been absolute recovery. Only a limited number of dilutions were therefore employed

TABLE XII

ACTION UPON THE TONGUE

	2 PER CENT
Sodium bicarbonate	0
Calcium chloride	0
Potassium chloride	0
Potassium sulphate	0
Sodium sulphate	0
Magnesium sulphate	0
Potassium bicarbonate	0
Chloretone ½ per cent	0
Quinine urea hydrochloride	0
Novocaine	0
Cocaine	+11

so that the method would not become irksome and prolonged over an undesirable length of time.

Results.—The comparative activity of substances acting alone is shown in Table XII. None of the substances tested showed any mucous membrane anesthesia whatever, with the exception of cocaine. This promptly produced a numbness lasting eleven minutes, more marked on the under surface of the tongue. As in the tests upon the rabbit's cornea, the superiority of cocaine as a surface anesthetic was easily demonstrated.

TABLE XIII

ACTIVITY OF POTENTIATION MIXTURES

NOVOCAINE	TWO PER CENT
2% Magnesium sulphate	0
2% Sodium sulphate	0
2% Calcium chloride	0
2% Potassium chloride	0
2% Potassium sulphate	0
2% Quinine urea hydrochloride	0
½% Chloretone	0
2% Potassium bicarbonate	+8
2% Sodium bicarbonate	+11

As was to be expected, no anesthetic action was observed in mixtures of novocaine with the inert salts, potassium salts, chloretone and quinine urea hydrochloride. Very marked anesthetic effect was produced by equal mixtures of novocaine and the bicarbonates. It was demonstrated that novocaine plus bicarbonate roughly equals the same strength of cocaine, at least in the 2 per cent dilutions employed.

Discussion.—This method is the most accurate one employed thus far. There is not the question of individual variation. By using the tongue tip the influence of pressure and salivary dilution is avoided. These play a part when pledgets of cotton are placed in the buccomandibular sulcus. Anesthesia does not leave the tongue tip suddenly, but any inaccuracy that might result from this feature is obviated by the fact that all tests were made on the same individual over a period of several days so that he learned to use the same criteria of recovery in all cases.

VI-*a*. ANESTHESIA OF THE DOG'S SKIN BY DERMAL WHEALS

Subcutaneous infiltration in the dog has been employed by Pittinger[20] as a biologic method for the quantitative evaluation of local anesthetics. He injected 1 c.c. of solution at a single puncture and tested sensation in the overlying skin with a red hot wire. He claims to have determined that cocaine was eleven times as active as novocaine by this method. It has long been pointed out that subcutaneous tissues are not suitable for such experiments because they are not sufficiently sensitive, and since they are not situated on the surface of the body it is impossible to determine the disturbance of sensation from the substance injected but only that of the overlying skin. We have modified this procedure by substituting intracutaneous infiltration for subcutaneous injection. It is then possible to apply the test for sensation at the exact point where the solution exerts its action.

Method.—The hair was closely clipped from an area on the dog's back, then removed completely by dissolving with barium sulphide depilatory mixture. Dermal wheals one-half inch in diameter were then raised in this area, using the Meeker regional anesthesia syringe and finest hypodermic needle. One-fourth of a cubic centimeter of solution was employed in each wheal and extreme care taken so that the infiltration was entirely intracutaneous. By means of a red hot wire the wheals were then tested at intervals for the return of sensation.

Results.—To those who have had experience in animal experimentation the difficulty in interpretation of sensations is well known. It is obvious that such results are only of questionable value. The data expressed in Tables

TABLE XIV

ANESTHETIC VALUE OF SUBSTANCES ACTING ALONE

	2%	1%	½%	
Distilled water				11
0.9% salt solution				5
Magnesium sulphate		5		
Sodium sulphate		10		
Calcium chloride		10		
Gelatine	15			
Potassium chloride		8		
Potassium sulphate		15		
Potassium bicarbonate		10		
Sodium bicarbonate		15		
Novocaine		18		
Cocaine		30		
Quinine urea hydrochloride				

TABLE XV

ANESTHETIC VALUE OF POTENTIATION MIXTURES

	1% EACH
Novocaine and gelatine	24
Novocaine and sodium bicarbonate	20
Novocaine and chloretone (½%)	22
Novocaine and quinine urea hydrochloride	32
Novocaine and magnesium sulphate	15
Novocaine and potassium chloride	22
Novocaine and potassium bicarbonate	24
Novocaine and potassium sulphate	19
Novocaine and sodium sulphate	15

XIV and XV represent experiments performed upon twelve dogs. Only gentle dogs were selected. They were not tied down or restrained in any manner, but were usually blindfolded, held upon the lap of an assistant and kept quiet by petting. No restraint was necessary even during the intracutaneous injection. Evidence of pain was manifested in a variety of ways, such as straining, whining, wiggling or shaking the skin by action of the platysma muscle. The most reliable manifestation was that of shaking the skin or wincing, while the animal remained otherwise quiet. These arbitrary manifestations were extremely variable, difficult to interpret and inaccurate. For example, dog No. 3 was very tame and lay quietly in an assistant's lap. All wheals were raised without any resistance. When stimulation over a wheal of salt solution produced no pain he was stimulated in normal skin. He tolerated the burn just as well in unanesthetized skin as in a

wheal of novocaine, until almost the entire thickness of the skin was burned through. He was then less valuable for subsequent tests because one could not tell whether he complained at stimulation of a wheal or at pain from the first burn in unanesthetized skin.

Dog 11 was of a different nature. Wheals were raised during considerable struggling. Later he winced and struggled at all forms of stimulation, even touch with a lead pencil. When a fresh wheal of cocaine was stimulated with the red hot wire he cried out loudly, although he could not possibly have felt any other sensation than moderate pressure. In another dog wheals regarded sensitive ten minutes after infiltration were repeated and found to be entirely insensitive twenty minutes after infiltration. These are a few of the difficulties encountered in trying to interpret a dog's sensations.

Discussion.—While experimentation on dogs is of value in the determination of local irritation and toxicity of anesthetic drugs, it furnishes very questionable data on painful sensations. A dog may either suffer patiently without external manifestation or struggle, strain, whine or cry out in dissatisfaction at its treatment similar to manifestations of actual pain. Such actions are more likely to occur when the animal is being fussed over. Rather than consider this method one of accurate pharmacologic assay we maintain that it has exceedingly little value. While Pittinger determined cocaine to be eleven times as powerful in anesthetic value as novocaine we were usually unable to distinguish novocaine from cocaine. His results are entirely at variance with those of other investigators using the human skin, so that we can only conclude that the method should be discarded as erroneous for quantitative determinations.

VI-*b*. ANESTHESIA OF HUMAN SKIN BY DERMAL WHEALS

Anesthesia of human skin by dermal wheals is an attractive method because it is identical to clinical usage. The patient is allowed to judge in all cases and interpret his own sensations. The great advantage of this method over others was first emphasized by Braun, who determined anesthetic strength both by duration of anesthesia and by minimal effective concentration. It has been shown by several surgeons to give the nearest approach to absolute anesthetic power.

Method.—The thighs and anterior abdominal wall were closely shaved. Dermal wheals were then raised with the Meeker syringe and finest hypodermic needles freshly sharpened. The needle was thrust beneath the epidermis parallel to the skin surface with the bevel downward. At the moment the point of the needle was buried in the epidermis injection began, which was always endermic and not subcutaneous. Dermal wheals were thus made to stand up from the surrounding skin surface like an urticarial wheal. Wheals were one-half of an inch in diameter and required one-fourth of a cubic centimeter of solution each. It is important that all wheals be as nearly as possible the same size and contain the same amount of solution, all of which has been injected intracutaneously. It is also necessary that adequate control wheals of salt solution and of novocaine be tested together with the

unknown mixture, otherwise a distorted sensation might be interpreted as anesthesia.

All wheals were made upon the author by himself, most of them on the anterior surfaces of the thighs from knees to hips. The skin in this region is of sufficient thickness that wheals may be raised painlessly when the substance is anesthetic. The sensitiveness of the skin as well as the rapidity of absorption varies in different areas of the body. It also varies in different individuals, depending upon familial traits, exposure and muscular exercise. By employing the same skin areas in the same individual these factors remained constant. The duration of anesthesia in the same cutaneous area may also be shortened by previous brisk massage, heating or muscular exercise because of the improved circulation and consequent rapid absorption. In these tests the patient remained seated and sources of external heat were avoided. Wheals were marked with a circle of mercurochrome as soon as raised so that the center of the endermic infiltration was easily identified after the wheal had disappeared.

TABLE XVI

ANESTHETIC VALUE OF SUBSTANCES ACTING SEPARATELY

	AQ. DIST.		PHYSIOL. SALT SOLUTION								
	1%	½%	1%	½%	¼%	⅛%	1/16%	1/32%	1/64%	1/128%	
Physiologic salt solution											0
0.45% salt solution											0
Distilled water											0
Magnesium sulphate	0		0	0							
Sodium sulphate	0		0	0	0						
Sodium bicarbonate	0		0	0							
Calcium chloride	18		25	3?	0						
Potassium bicarbonate	18		27	3?	0						
Potassium sulphate	20		28	5?	0						
Potassium chloride	19		25	5?	0						
Gelatine	15		0	0							
Chloretone		28		18	5?	0					
Quinine urea hydrochloride	160		95	52	40	28	11?	0	0		
Novocaine	33		28	20	15	8	5	0			
Cocaine	45		32	27	20	16	12	8	5?	0	

Other investigators have tested for anesthesia by means of a fine cotton wisp. The stroking of the area with a wooden applicator or toothpick, or scratching with a needle as in vaccination was found to be more accurate. By means of the cotton wisp disturbed or distorted sensation as in a wheal of physiologic salt solution may be mistaken for anesthesia. Of much greater importance than the manner of testing is the employment of adequate control wheals with the series of dilutions of the unknown solution.

Results.—Table XVI expresses the action of each substance alone. Magnesium sulphate, sodium sulphate and sodium bicarbonate possess no anesthetic value but are similar in effect to wheals of physiologic salt solution. Calcium chloride was found to be anesthetic in 1 per cent solution, although all previous tests had shown it to be inert. The three potassium salts are about equal in anesthetic action. In previous tests they were almost the equal of novocaine, but in dermal wheals they are much inferior. Gelatine was anesthetic in distilled water but not in salt solution. Chloretone was

inferior to novocaine and cocaine, while quinine urea hydrochloride was the superior in higher concentrations.

There was no potentiation with any of the mixtures tried (Table XVII). The duration of anesthesia with the mixtures was approximately the equal of the same strength of novocaine acting alone. There was not even summation of action with potassium salts in ½ per cent strength, and the addition of bicarbonate did not appreciably improve the solution. Chloretone and novocaine give summation only. The addition of quinine urea hydrochloride prolongs anesthesia by imperfect summation and not potentiation.

While the specific action of a substance should be studied when the solution is isotonic, valuable information is also obtained when distilled water is used as the solvent. Studied in this manner many substances are decidedly painful before their anesthetic action is established, although the true local anesthetics as novocaine and cocaine in strengths sufficient to anesthetize are entirely painless to infiltration. An anesthetic producing pain for its injection has been called "anesthetica dolorosa." Distilled water is very painful and only partially anesthetic for a short period. Some surgeons claim to have performed operations painlessly by means of copious injections of water but certainly the very painful infiltration and tumefaction with imperfect anesthesia must have afforded but little more palliation than operation without any anesthesia whatever. The fact that injections of water have been observed to bring about good results in such painful affections as sciatica and neuralgia may be explained upon another basis than the supposed nerve blocking properties of the water.

An attempt to group these substances according to the amount of pain produced shows the following relationship:

TABLE XVII

ANESTHETIC VALUE OF POTENTIATION MIXTURES

	½%	¼%	⅛%	1/16%
Novocaine and sodium sulphate	19	14	9?	
Novocaine and magnesium sulphate	20	17	6?	
Novocaine and calcium chloride	20	18	11?	
Novocaine and potassium chloride	21	17	10?	
Novocaine and potassium sulphate	18	13	9?	
Novocaine and potassium bicarbonate	18	7	5	
Novocaine and sodium bicarbonate	21	15	11?	
Novocaine and chloretone		17	12	6?
Novocaine and quinine urea hydrochloride	64	40	27	18

1% Cocaine	0.
1% Novocaine	0.
1% Quinine urea hydrochloride	+++.
½% Chloretone	++.
1% Gelatine	++++.
1% Potassium chloride	++++.
1% Potassium sulphate	++++.
1% Potassium bicarbonate	++++.
1% Calcium chloride	+.
1% Sodium sulphate	++.
1% Sodium bicarbonate	++.
1% Magnesium sulphate	+.
.9% Sodium chloride	+.
.45% Sodium chloride	++.
Distilled water	++++.

Potassium salts and quinine urea hydrochloride were very painful before anesthetic, thus showing the necessity for the same tonicity of the fluid injected as that of the tissues.

To determine the influence of gelatine, dilutions of novocaine were used in a 4 per cent gelatine medium. Injections were made in three series. In one the gelatine was sterilized by boiling for five minutes. In the two remaining series the mixtures were not boiled. One of these was physiologic salt solution and the other distilled water. The three series were approximately equally anesthetic and only questionably superior to the action of novocaine alone. Absorption was not appreciably delayed by the use of gelatine. (Table XVIII.) This confirms and extends Braun's observations with sterilized gelatine solutions. Even though gelatine improved the novocaine solution, the fact that it is an excellent bacteriologic culture medium and congeals in solutions above 2 per cent at room temperature, would prohibit its use as adjuvant to local anesthesia.

TABLE XVIII

ACTION OF NOVOCAINE IN GELATINE SOLUTION

NOVOCAINE	½%	¼%	⅛%	⅟₁₆%
4% gelatine in salt sol. sterilized by boiling	24	19	11	5?
4% gelatine in salt sol. unsterilized	25	20	17	13?
4% gelatine in aq. dist. unsterilized	25	21	13	6?

TABLE XIX

INFLUENCE OF ADRENALIN UPON ANESTHETIC ACTIVITY

	1%	½%	¼%	⅛%	⅟₁₆%	⅟₃₂%
Novocaine	28	20	15	8	5	0
Novocaine and adrenalin (10 drops per 100 c.c.)		120	82	43	10	?

The addition of ten drops of adrenalin (Parke Davis, 1 : 1000) to the hundred c.c. of anesthetic solution prolongs anesthesia in a very decisive manner. There is not true potentiation because the minimal effective concentration is not altered. (Table XIX.) The effect has been repeatedly shown to be due to the vasoconstriction of the adrenalin which has earned it the name of the "chemical tourniquet." The value of this agent is universally recognized, so that the only excuse for including it in this series of observations is for comparison with the other drugs employed for synergistic action.

Discussion.—This method involves direct action on the terminal nerve filaments and sensory end organs. The complicating factor of penetration is not present, since the force in injection produces diffusion of the solution. In well-formed wheals the solution is confined to the upper layers of the skin and is never below the derma proper. Anesthesia does not extend beyond the limits of the raised margin, and sensation returns from the periphery toward the center. When wheals are accurately placed, a sufficient number of controls employed and when the patient is experienced in the interpretation of the end point, this method is easily the most valuable one for quantitative estimation of local anesthetic power.

GENERAL DISCUSSION

A comparison of results obtained by the different methods shows that there is considerable variation. Determinations of local anesthetic action by the methods employing decapitated frogs must always be discarded when they do not agree with data on the human.[19] In addition to the great difference of station in the scale of animal life is the fact that experimental conditions do not correspond to those of clinical usage. The action of the frog's sciatic nerve has been compared to nerve blocking in surgery. But the frog is first killed, the nerve trunk isolated, immersed in the anesthetic solution and the end point determined by motor response. There is not the problem of diffusion of the solution through tissues surrounding the nerve trunk and the influence of anemia, pressure and edema, and the absorption of the anesthetic medium, all of which are of great importance in clinical usage.

The second method, the action upon the sensory fibers of the lumbosacral plexus is open to many of the same objections. The fact that the frogs were first killed and that the reflex arc had to be employed in determining the presence of sensation render it worthless when results do not agree with those obtained upon man.

The third method, anesthesia of the frog's skin, is not to be compared to mucous membrane anesthesia in man. It has been maintained that because the frog's skin is wet, conditions of mucous membrane anesthesia in man are closely reproduced. As a matter of fact there is a vast difference both anatomically and physiologically which would indicate a different permeability ratio. By this method chloretone was far the superior of cocaine, while it is of no value at all in surface anesthesia in man. As a sole method of evaluating local anesthetics, killed frogs would give erroneous data. Their continued use for this purpose represents merely a heritage from older physiology.

All three experiments on frogs showed potentiation with potassium and alkalies, more marked with the latter. Potentiation with alkali was also quite marked in surface anesthesia both on the cornea of the rabbit and on the human tongue. These methods are identical to clinical use and suggest the addition of alkali to novocaine solutions for mucous membrane anesthesia. The author plans to give the method a clinical trial particularly in urethral anesthesia.

It seems certain that the improvement of alkali consists in increased ability to penetrate mucous membranes. This same power of penetration would be of immense value in nerve block and field block. In field block fanwise injections are made in such a manner in the same plane that intervening tissues are penetrated by the solution to form a continuous wall interrupting conductivity of all nerve filaments passing through it. While there is no direct experimental data bearing on this procedure, results with dermal wheals as well as clinical experience indicate no benefit from the addition of alkali. Granting that free bases penetrate more readily than the salts Sollman states that in surgical use the anesthetics do not reach the nerve filaments as pure salts, but that they are partly converted into carbon-

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EFFECT OF CHLORINE TREATMENT AS GIVEN AT EDGEWOOD ARSENAL, MARYLAND, ON THE LEUCOCYTE COUNT*

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VEDDER and Sawyer,[1] in explaining the beneficial effects of chlorine on common colds, whooping cough, and certain other respiratory infections of the upper respiratory tract, attributed at least part of this curative action to an increase of polymorphonuclear leucocytes in the affected mucous membranes and to a stimulation of phagocytosis.

An attempt was therefore made to determine whether the administration of chlorine caused a change in the leucocytes. Since the taking of blood from the mucous membranes of the respiratory tract is scarcely practicable, the work was confined to leucocyte counts of the peripheral blood.

The chlorine treatment was given twice a day, at 10:00 A.M. and at 1:00 P.M. in a large wooden chamber accommodating twenty people. The mixture of air and chlorine was drawn through the chamber at a rate to give a concentration of between 0.014 and 0.015 mg. per liter of air. The ordinary treatment was for one hour. A leucocyte count and a smear for a differential count were made on patients immediately before they entered the chlorine chamber, immediately after the hour's treatment and as nearly as possible one hour later. Counts were made on all persons available for this third count. They included sufferers from acute coryza, bronchitis, sinusitis and a few normal persons. The data were collected over a period of six months, from September to February, the largest number of cases being secured in the winter months. One hundred cases were studied.

The leucocyte count in 25 cases of whooping cough was also studied, but as these patients came from some distance to take the treatment, they were not available for a third count and only the counts immediately before and immediately after the treatment could be secured.

SUMMARY

1. From the tables it may be seen that in 68 per cent of the cases of cold or mild influenza the second count, that immediately following the exposure, was lower than the initial count; in 27 per cent of the cases it was higher; in 4 per cent it was the same as the first count. In 89 per cent of the cases the leucocyte counts were made on patients taking the treatment in the afternoon. The counts made in the morning showed a drop in only 50 per cent of the cases. This suggests that the larger number of decreased counts in the afternoon cases might be due to a natural subsidence from an initial digestive leucocytosis. Forty-eight per cent of all counts, however, showed a drop of over 1000, which should eliminate physiologic factors. It was in

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TABLE I

LEUCOCYTE AND DIFFERENTIAL COUNTS IN CHLORINE TREATMENT OF SOME RESPIRATORY DISEASES

	LEUCOCYTE COUNT					DIFFERENTIAL COUNT (ORDER OF CELLS FOLLOWED IN DIFFERENTIAL COUNT: POLYMORPHONUCLEARS, LYMPHOCYTES, MONONUCLEARS, EOSINOPHILES, BASOPHILES.)		
	Date	Hour	Before	After	One Hour Later	Before	After	One Hour Later
A	8-23	9	4,800	4,200	4,300			
D	8-26	1:10	8,300	8,200	9,400			
A	"	"	7,000	6,400	8,000			
E	8-29	"	12,000	10,600	18,400			
A	"	"	5,200	6,000	6,800			
L	9-9	"	8,000	6,800	6,600			
L	9-10	"	8,600	8,000	8,000			
S	"	"	9,600	8,800	9,800			
V	"	"	10,400	6,800	8,300			
V	9-15	"	9,800	7,000	7,000			
S	9-17	"	9,700	8,200	8,800			
M	9-26	"	6,600	4,200	5,000			
A	10-10	"	6,800	7,400	6,600			
S	10-22	"	9,200	9,600	8,600			
W	10-23	"	7,800	8,000	8,000			
S	10-24	"	8,600	8,000	8,200	(54-35-6-4-1)	(69-21-4-5-0)	(60-30-4-6-0)
M	10-24	"	7,200	8,500	8,700			
M	11-6	"	10,600	10,600	10,000			
E	"	"	6,500	6,100	7,000			
D	11-13	"	7,200	6,600	6,800			
N	"	"	9,300	9,700	12,600			
N	11-14	"	10,000	9,600	11,000			
K	"	"	8,600	8,000	6,800			
C	"	"	8,600	10,200	9,600			
D	11-17	"	11,200	12,000	8,800			
D	11-18	"	10,200	8,800	7,400			
S	11-19	"	8,000	8,400	6,600			
W	11-21	"	7,800	7,600	9,200			
K	"	"	4,600	4,600	5,000	(52-33-7-6-1)	(56-31-9-3-0)	(58-32-3-5-1)
S	11-22	"	8,600	6,000	8,260			
A	"	"	9,600	10,600	9,600			
O	"	"	5,400	5,000	6,600			
R	11-24	"	7,000	6,000	7,600			

TABLE I—CONT'D

LEUCOCYTE AND DIFFERENTIAL COUNTS IN CHLORINE TREATMENT OF SOME RESPIRATORY DISEASES

	LEUCOCYTE COUNT					DIFFERENTIAL COUNT (ORDER OF CELLS FOLLOWED IN DIFFERENTIAL COUNT: POLYMORPHONUCLEARS, LYMPHOCYTES, MONONUCLEARS, EOSINOPHILES, BASOPHILES.)		
	Date	Hour	Before	After	One Hour Later	Before	After	ONE HOUR LATER
A	11-24	1:10	6,900	8,000	8,000			
H	"	"	8,200	10,000	8,800			
D	11-26	10:15	8,600	8,200	8,600	(63-28-7-1-0)	(63-31-4-1-1)	(67-30-2-0-1)
S	"	"	8,800	10,200	10,000			
A	"	"	7,400	8,200	10,000	(53-24-20-1-2)	(62-28-10-0-0)	(65-26-8-1-0)
L	11-28	10:00	6,200	5,500	7,400	(63-30-6-1-0)	(64-24-11-1-.3)	(67-22-9-1-1)
P	"	1:30	8,900	8,000	8,200			
C	12-1	"	7,600	7,000	9,400			
W	"	"	10,400	8,400	13,000			
W	12-2	10:00	14,000	12,200	10,000			
C	"	1:30	9,200	6,200	9,600			
P	"	2:00	8,800	5,000	7,800	(70-16-11-2-0)	(72-12-14-1-1)	(55-32-12-1-0)
W	12-3	1:30	8,400	8,200	6,800	(67-25-6-5-1)	(63-15-20-0-2)	
P	12-4	1:30	7,000	8,400	8,000			
M	"	"	6,600	7,600	6,800			
A	"	10:00	6,600	8,400	10,000			
A	12-5	11:00	8,200	9,600	9,800			
R	12-8	1:15	17,000	9,000	9,000	(67-28-4-.5-1)	(66-30-2-2-.4)	(58-40-1-.5-.5)
M	"	"	14,200	8,200	7,800	(57-38-4-0-1)	(64-32-3-.5-.5)	(63-32-3-0-2)
H	12-10	1:30	22,000	8,300	16,800	(56-28.5-14-.5-1)	(61-30-8-1-0)	(61-31-7.5-0-.5)
E	12-12	"	22,400	7,000	7,400	(68-16-15-0-1)	(67-21-9-1-1)	(62-22-9-5-1)
C	"	"	18,800	9,800	9,200	(78.4-18-3.6-0-0)	(60-30-10)	(67-21-11-0-.3)
S	"	"	7,400	7,200	6,400	(67-26-6.7-0-.3)	(70-17-10-2-0)	(68-11-19-1-1)
C	12-13	"	8,000	8,200	9,400	(80-16-4-0-0)	(69-19-12-0-1)	(72-20-7.5-0-.5)
B	"	"	10,400	7,400	7,800	(66-27-6-0-1)	(77-9-12-0-1)	(70-24-4-0-2)
B	12-15	"	11,200	9,600	11,900	(78-15-7-0-.5)	(76-17-5-.5-.5)	
B	12-16	"	11,000	9,400	7,000	(73-22-3-.5-.5)	(75-18.5-4.5-1-.5)	(75-22-1-1-.5)
H	12-16	"	10,800	7,600	8,600	(68-26-5-0-1)	(66-29-2-3-0)	(71-25-4-0-0)
M	"	"	8,000	11,000	7,000	(59-29-10-1-1)	(57-31-10-1-1)	(61-30-6-2-1)
N	12-17	"	16,000	8,000	9,600	(69-20-8-2-1)	(69-25-5-1-0)	(62-29.5-6-2-.6)
L	12-18	"	16,000	11,000	14,000	(66-27-5-1-1)	(64-25.5-9-1-.5)	(65-27-7-.5-.5)
E	"	"	23,200	8,600	16,000	(58-27-13-1-0)	(69-18-12-.5-.5)	(55-26-17-1-0)
N	"	"	13,400	7,000	12,800	(62-24-11-1-1)		(55-32.5-11-1-.5)
W	12-19	1:20	12,600	12,600	10,600	(67-27-6-0-0)	(72-23-4-.3-0)	(61-34-5-.3-0)

TABLE I—CONT'D

LEUCOCYTE AND DIFFERENTIAL COUNTS IN CHLORINE TREATMENT OF SOME RESPIRATORY DISEASES

	LEUCOCYTE COUNT					DIFFERENTIAL COUNT (ORDER OF CELLS FOLLOWED IN DIFFERENTIAL COUNT: POLYMORPHONUCLEARS, LYMPHOCYTES, MONONUCLEARS, EOSINOPHILES, BASOPHILES.)		
	Date	Hour	Before	After	One Hour Later	Before	After	One Hour Later
A	12-27	1:20	15,600	14,800	14,400	(60-32-7-0-.3)	(61-30-7.5-.5-0)	(66-26-7-.5-0)
W	12 29	1:40	8,000	8,000	6,400	(71-21-7-.3-1)	(63-27-7-2-.3)	(61-29-9-1-0)
A	12-31	1:30	8,200	8,600	9,000	(62-32-5-0-0)	(53-40-6-0-.2)	(54-39-7-0-.3)
A	12-30	1:45	20,200	19,400	13,200	(63-27-9-0-.3)	(75-14-10-.5-0)	(70-25-4-0-0)
A	1-5	1:30	8,600	6,800	6,900	(66-26-7-0-1)	(69-25-5-0-.2)	(62-31-6-0-.3)
M	1-5	2:00	15,400	15,000	13,400	(72-18-7-3-.3)	(80-14-6-.3-0)	(77-15-5-3-0)
A	1-6	1:30	7,800	6,600	7,800	(69-25-5-1-0)	(70-20-9-1-0)	(67-24-7-1-1)
M	"	2:00	12,200	14,200	16,600	(66-22-8-4-0)	(73-17-7-3-0)	(67-20-9-3-.3)
C	1-8	"	6,400	8,200	9,800	(52-42-4-2-0)	(54-40-5-1-0)	(53-40-6-.4-.6)
D	"	"	6,100	10,000	7,600	(57-32-3-5-2)	(50-40-9-0-.5)	(57-28-11-3-1)
A	1-9	1:20	9,400	8,400	10,000	(72-20-7-0-1)	(73-20-6-.5-.2)	(76-19-4-.5-0)
F	"	"	14,200	11,400	7,200	(60-30-8-2-0)	(62-31-5-1-.3)	(60-32-6-2-0)
D	"	"	12,600	7,000	8,400	(64-31-5-.3-0)	(58-34-4-4-0)	(60-31-8-.3-.7)
C	"	"	12,200	10,800	8,800	(64-24-10-1-0)	(60-30-8-1-0)	(60-28-10-1.6-.4)
A	1-10	1:45	6,800	6,200	7,200	(70-23-6-.3-0)	(64-26-8-1.5-.3)	(64-27-8-.7-.3)
M	"	"	13,000	11,800	12,600	(71-24-1-4-0)	(75-15-5-3-1)	(72-18)
C	"	"	11,800	13,200	14,200	(79-14-4-0-2)	(73-18-8-.4-0)	(76-14-8,5-1)
W	1-13	"	14,600	13,800	10,600	(64-28-6-1-1)	(63-28-8-.3-0)	(64-26-9-.8-.2)
M	-1-13	"	8,600	7,400	7,000	(57-37-5-1-0)	(56-36-7-1-0)	(56-36-7-.5-.5)
M	1-17	"	17,200	14,400	16,000	(74-19-4-3-0)	(66-21-10-3-0)	(72-23-3-2-0)
W	1-23	2:00	11,800	10,400	12,400	(69-18-9-3-.3)	(74-21-5-0-0)	(76-17-5-1-0)
W	1-24	"	14,000	10,400	11,400	(60-27-9-2.5-.4)	(61-32-2-4-.6)	(57-35-6-2-.3)
S	1-31	1:30	13,200	9,000	8,200			
S	2-2	"	10,800	6,800	7,000	(57-38-3-1-.5)	(51-40-7-1-0)	(51-37-10-1.5-0)
S	2-4	"	7,600	5,200	5,000			
A	2-5	1:15	6,000	6,000	5,300			
D	2-5	1:15	17,000	14,000	14,000	(72-23-4-0-1)	(77-22-1-0-0)	(66-28-6-0-0)
W	2-6	9:45	6,400	7,200	8,200	(50-38-9-1-1)	(54-39-5-2-0)	(47-43-8-1-.2)
D	2-7	1:50	19,000	15,000	11,000	(85-11-3-.6-0)	(85-13-1.8-0-0)	(79-16-4-1-.1)
M	2-9	10:00	10,400	10,000	8,200			
D	2-11	10:45	10,800	13,000	11,400	(65-26-6-2-0)	(57-38-4-1-0)	(60-30-8-2-0)
A	2-18	1:30	7,600	7,200	8,600	(72-25-2-.3:0)	(71-23-5-.3-.3)	
M	2-26	"	9,000	6,200	7,000			

TABLE II

WHOOPING COUGH CASES

DATE	PATIENT	HOUR	BEFORE Leucocyte Count	BEFORE Differential Count	AFTER Leucocyte Count	AFTER Differential Count
Nov. 28	S. M.	2:10	11,600	(60-40)	10,600	(75-25)
Dec. 1	" "	"	13,400	(78-22)	12,000	(62-38)
Nov. 29	N. M.	"	14,900	(70-30)	11,800	(70-30)
Dec. 1	" "	"	15,000	(55-45)	13,800	(77-23)
Nov. 29	M. M.	"	20,600	(33-66)	12,000	(30-70)
Dec. 1	" "	"	23,800	(55-45)	15,800	(42-58)
Dec. 8	P. M.	1:30	27,800	(17-72-9-1-1)	20,400	(12-83-2-1-1)
Dec. 8	J. M.	"	18,400	(35-63-2-0-0)	18,600	(16-80-1 5-1-1.5)
Dec. 8	P. M.	"	40,400	(25-65-5-5-0)	36,400	(40-56-3-.5-0)
Dec. 8	F. M.	"	21,600	(54-38-6-1-1)	16,000	(28-70-2-0-0)
Dec. 9	" "	"	14,800	(50-44-6-0-0)	11,400	(25-62-12 5-.5-0)
Dec. 9	P. M.	"	26,200	(39-60-0-1-0)	31,800	(38-54-4.5-3.5-0)
Dec. 9	" "	"	16,400	(15-59-25-1-0)	21,800	(16-73-9-1-1)
Dec. 9	R. B.	"	22,000	(31-61-4-3.5-.5)	16,000	(27-65-4-3.5-5)
Dec. 10	" "	"	17,800	(45-49-6 0-0)	16,800	(42-51-6 0-.5)
Dec. 15	M. H.	2:00	33,800	(60-35-3-0-1)	32,000	(58-34-7 0-0)
Dec. 17	R. B.	"	16,000	(40-52-8-0-.5)	13,400	(43-42-12-1-.2)
Dec. 19	M. H.	"	28,000	(34-62-3-1-0)	19,000	(32-57-10-0-.5)
Dec. 19	B. L.	"	22,000	(33-52-13-1-.5)	17,000	(42-49 7-.7 3)
Dec. 20	R. B.	2:10	19,500	(37-51-12-0-0)	16,000	(54-37-9 0-3)
Dec. 20	B. L.	1:45	15,000	(44-49-6-1-.5)	12,300	(40-52-7-.3-0)
Jan. 21	E. S.	2:00	24,500	(40-54-5-.6-0)	21,500	(39 52-7-4-2)
Feb. 19	W. C.	11:20	16,000	(34-57-5-3-1)	14,400	(31-61-4-3-1)
Feb. 19	N. S.	"	26,000	(34-60-5-1-0)	26,000	(32-58-5-4-0)
Feb. 19	R. S.	"	14,200	(64-28-4-4-.5)	13,200	(48-37 6 4-3)

the cases showing a leucocytosis originally that the fall was most marked and in 85 per cent of the cases having an original count of over 10,000 there was a decrease, averaging 4,000. The differential count was only slightly changed, 27 cases having a rise in polymorphonuclears, 23 cases showing a drop and 3 showing no change in polymorphonuclears.

2. Of the 25 cases of whooping cough, 20 or 80 per cent showed a decrease in total leucocytes after the hour of treatment. The average count in these cases was 20,465 before treatment and 15,920 after treatment, or an average decrease of 4,545. Of these cases 17 showed a drop in polymorphonuclear leucocytes averaging 8.9 per cent.

DISCUSSION

The interpretation of these changes is by no means certain, yet it seems probable that the decrease in the peripheral blood may be caused by the migration of leucocytes to the point of irritation, namely, the mucous membranes of the respiratory tract. The migration of from 2000 to 4000 leucocytes in each c.c. of blood to this limited area would lead to a concentration of leucocytes in these parts which should be beneficial to the patient. However, there was no directly apparent connection between the extent of the drop and the degree of improvement. This is undoubtedly to be explained by the fact that the action of chlorine is very complex, and that the increase of leucocytes to the affected part, if it occurs, is only one of five or six factors concerned in the curative action.

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LABORATORY METHODS

A CAGE DEVICE FOR THE STUDY OF KETOSIS AND NITROGEN METABOLISM IN SMALL ANIMALS*

By Harold Levine, B.S., and Arthur H. Smith, Ph.D., New Haven, Conn.

In metabolism studies on rats recorded in the literature,[1-9] various devices and types of cage have been used to collect the urine. In most instances, the animal rests on a wire screen in a cage, the mesh of which is large enough to permit the urine to drop through and trickle down the sides of a large glass funnel, into a receiving flask placed just beneath the stem of the funnel. By washing down the funnel several times daily, the accuracy of this method of collection is increased. In the apparatus used by Mitchell,[10] the urine drops through a wire screen and is absorbed by filter paper placed in a large crystallizing dish which serves as the bottom of the cage. The above mentioned methods, obviously, are not suited for a study involving a volatile urinary constituent such as acetone. In an effort to surmount this difficulty, a new type of cage, described in this paper, was devised.

Acetone will not volatilize when held beneath a layer of mineral oil. An acetone solution of known concentration was allowed to remain in the cage for a period of six days under a layer of mineral oil five inches in depth. At the end of this period, the acetone solution was analyzed and the result showed that no loss occurred either through volatilization or absorption by the oil. In the cage described below, the principle of urinary collection depends on the capacity of the oil to prevent loss of acetone and on the greater density of the urine as contrasted with the oil.

DESCRIPTION OF CAGE AND METHOD OF COLLECTION OF URINE

Cage Proper.—A diagram of the cage proper is shown in Fig. 1, Part I, with both the feeding (*A*) and drinking (*E*) devices attached. It is cylindrical in shape, 8½″ high and 8¾″ in diameter and constructed of galvanized iron. Ventilation is provided by means of a ¼″ mesh wire screen (*G*) soldered on ½″ from the top of the cage. Small holes (not shown in the diagram, see Fig. 2) ¼″ in diameter, placed at intervals around the cage, 4½″ from the top of the cage, also serve to give proper ventilation.

The feeding device (*A*) consists of a beaker, inside of which a glass food cup (*B*) rests on a rubber stopper (*C*). This beaker is supported by means of a metal ring (*D*), 2½″ in diameter, attached to the side 5¾″ from the top of the cage. In order to have a minimum of scattering by the rat, the food

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receptacle proper (B) is placed about 1″ from the top of the beaker. Experiments have shown that rats, as a rule, do not defecate or urinate in this feeding device.

The animal obtains water by means of an inverted bottle (E) which rests on the wire screen (G) at the top of the cage. From this bottle, a glass tube extends downward into the cage. By licking the end of the tube, the animal receives its water supply.

Urine Collecting Device.—The lower part of the cage which serves to effect both the collection of urine and its separation from feces is shown in Fig. 1, Part II. This funnel-shaped section of the apparatus is also con-

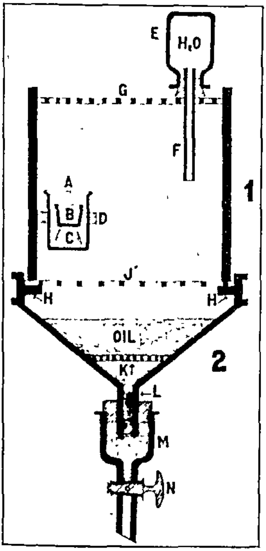

Fig. 1.

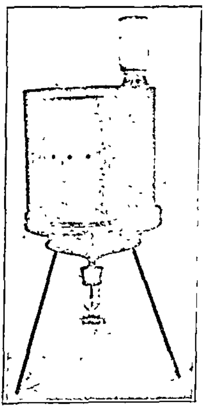

Fig. 2.

Fig. 1.—Diagram of metabolism cage (⅓ actual size).

Part 1. *A*, feeding device; *B*, food cup; *C*, rubber stopper; *D*, metal ring; *E*, water bottle; *G*, screen.

Part 2. *H*, lugs to support screen (*J*) and Part 1; *J*, screen on which animal rests (allows both urine and feces to drop through); *K*, screen to retain feces; *L*, stem; *M*, separatory funnel; *N*, stopcock.

structed of galvanized iron, with a diameter of 9″ across its top. The upper cylindrical cage (Part 1) fits snugly into it, resting on four small lugs (H, H). Between the upper and lower parts of the cage is a wire screen (J) which supports the animal. The mesh of this screen is large enough, ⅜″ mesh, to permit both the urine and feces to drop through into the mineral oil directly below.

The sides of the funnel taper down, with an angle approximately 30°, to the stem (L) 4½″ in length. This stem, with an inside diameter of ½″,

leads into the dropping funnel (*M*) which is attached by means of a tightly-fitting rubber stopper.

Mineral oil fills the entire funnel up to a level ½″ from the wire screen (*J*). Urine and feces drop through this screen, through the mineral oil as far as the small wire screen (*K*), which in turn retains the feces and allows the urine to flow down into the funnel (*M*), which contains a few cubic centimeters of 3 per cent sodium fluoride as preservative.

Collection of Urine.—At the end of the experimental period—usually of four days' duration—the urine is run off by means of the stopcock (*N*). To increase the accuracy of collection, a small glass plate is placed on the scale when the animals are to be weighed. Any urine voided during weighing can then be washed back into the cage. Inasmuch as no urine stains were observed on the sides of the upper section (I), only the screen (*J*) and the converging sides of the lower section (II) need be washed down with distilled water in an effort to obtain the last traces of urine. Finally, the washings are run off and combined with the urine sample which is kept in the refrigerator until

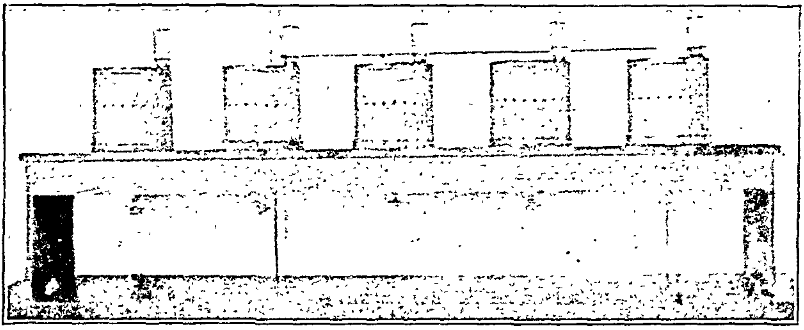

Fig. 3.

ready for analysis. After removal of the urine and washings, the mineral oil is drawn off, filtered from feces, sterilized, and used again.

Fig. 2 shows the cage in assembled form. In actual experiments with animals, a series of five cages is kept in a long wooden section (Fig. 3) having holes, 8¾″ in diameter, into which each cage fits closely.

Adherence to the measurements of the cage as given is necessary for its successful use in metabolism experiments.

Tests of Accuracy.—It is apparent that if the device described is to be used in metabolism experiments, the degree of accuracy of the cage in the collection of various urinary constituents must first be determined.

Tables I and II show the efficiency of the cage using acetone, nitrogen and a mixture of "acetone bodies" as test substances. Acetone was determined by the method of Van Slyke[11] and nitrogen by the Kjeldahl method. In all the experiments, the solution to be tested was trickled down the sides of the lower section (Fig. 1, Part II) and allowed to remain in the apparatus for four days. At the end of this period, the solution was run off and, together with the washings, was analyzed.

It was desirable first of all to ascertain the efficiency of the mineral oil itself in preventing volatilization of acetone. In Experiment 1, therefore, the

screen (J) was removed from the cage and an acetone solution was trickled down through the oil alone. This experiment shows clearly that no acetone was lost, testifying to the efficacy of the oil in the collection of urinary acetone.

Experiment 1 demonstrates the capacity of the oil to prevent volatilization of acetone. In actual metabolism experiments, the animal rests on the screen (J) and it was deemed advisable to determine what loss of acetone, if any, might be due to the presence of the screen. Accordingly, an acetone solution was trickled down over the screen (J) and allowed to drip into the oil directly below. Experiment 2 shows that when this procedure was carried out, a relatively large loss of acetone occurred, whereas the data for Experiment 1 indicate complete recovery. This loss may therefore be attributed to volatilization due to the presence of the screen (J).

In metabolism experiments with the rat, however, the value of 19 per cent obtained in Experiment 2 must be discounted considerably, when it is realized that in the experimental procedure no attempt was made to imitate the rat's habit of urination. Instead, the acetone solution, was trickled from a pipette in such a manner that *all* parts of the screen (J) were wet with the fluid. The rat, on the other hand, probably does not behave in this manner, but remains stationary on the screen and urinates directly through its wide meshes. It is obvious, therefore, that Experiment 1 more nearly approximates the actual experimental condition and undoubtedly 19 per cent is not the true index of the usual error of the cage in the collection of acetone but rather represents a maximum value.

Experiments 1 and 2 were concerned with only one of the three "acetone bodies," i.e., acetone. It was desirable next to find out, firstly, what losses the other "acetone bodies," i.e., aceto-acetic acid and beta-hydroxybutyric acid, would undergo in the cage and, secondly, to ascertain what loss acetone itself would suffer when all three "acetone bodies" are present in one solution as they are in urine. In the experiment referred to in Table II a mixture of all three "acetone bodies," made up in the approximate proportions excreted by the rat* was used. The solution contained 30.3 mg. of "acetone bodies" as follows: 4.2 mg. acetone, 7.2 mg. aceto-acetic acid (expressed as acetone) and 18.9 mg. beta-hydroxybutyric acid (expressed as acetone). Such a mixture, when trickled over the screen (J) into the oil suffered a loss of 9 per cent. Further analysis of the data in Table II shows that of this loss, 5.7 per cent was due to acetone and aceto-acetic acid, whereas beta-hydroxybutyric acid was responsible for the remaining 3 3 per cent loss. The data in this experiment also show that the sum of the acetone plus aceto-acetic acid present in the mixture accounts for a loss of 14 per cent, whereas beta-hydroxybutyric acid suffered a loss of only 5 per cent. Here again, the values are probably maximal ones.

In Experiment 3 (Table I) a sample of dog's urine, when trickled showed a loss of 3.1 per cent in nitrogen. Osborne and Mendel[2] report losses of 10 per cent or more in the collection of urinary nitrogen. Mitchell[10] by his method obtains an error of 2 to 3 per cent. The small error obtained with

*Unpublished data from this laboratory.

TABLE I

TESTS OF ACCURACY OF URINARY COLLECTION
ACETONE AND NITROGEN

EXP. NO.	URINARY CONSTITUENT	AMOUNT USED MG.	AMOUNT RECOVERED MG.	RECOVERY PER CENT	LOSS PER CENT	REMARKS
1	Acetone	6.84	6.84	100	00.0	25 c.c. of solution used. Screen (*J*) removed.
2	Acetone	9.50	7.70	81	19.0	25 c.c. of solution used. Screen (*J*) in place.
3	Nitrogen	427.6	414.4	96.9	3.1	20 c.c. of dog's urine used. Screen (*J*) in place.

TABLE II

TESTS OF ACCURACY OF URINARY COLLECTION
"ACETONE BODIES"

DETERMINATION*	AMOUNT USED		AMOUNT RECOVERED mg.	PERCENTAGE RECOVERY		PERCENTAGE LOSS		REMARKS
	mg.	Per Cent of Total Acetone		Of Each Constituent	Of Total Acetone	Of Each Constituent	Of Total Acetone	
Total acetone	30.3	100	27.6		91		9	Used 40 c.c. of a solution containing: Acetone = 4.2 mg. Aceto-acetic Acid = 7.2 mg. Beta-hydroxybutyric Acid = 18.9 mg. "Total Acetone" = 30.3 mg. 62.3% of the "total acetone" bodies present as beta-hydroxybutyric acid.
Acetone + Aceto-acetic Acid	11.4	37.7	9.7	87	32	13	5.7	
Beta-hydroxybutric Acid	18.9	62.3	17.9	95	59	5	3.3	

*Results of determinations of "acetone bodies" are expressed as acetone.

the cage described above suggests its value in metabolism experiments involving urinary nitrogenous substances.

SUMMARY

1. A metabolism cage is described which permits the collection of the urine of the rat. This cage is suitable for the study of ketosis in small laboratory animals such as the rat.

2. In the collection of urine, loss of volatile urinary substances is prevented by the use of mineral oil under which the urine is held.

3. The degree of accuracy in the collection of urinary "acetone bodies," i.e., acetone, aceto-acetic acid and beta-hydroxybutyric acid, as well as urinary nitrogen has been determined.

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STUDIES IN LOCAL ANESTHESIA. II

The Pharmacology of Some Para-Amino-Benzoate Compounds*

By H. McGuigan, S. J. Cohen, W. J. R. Heinekamp, et al., Chicago

INTRODUCTION

THE following series of para-amino-benzoate preparations were prepared and furnished to us by Roger Adams of the Department of Chemistry of the University of Illinois.

Series A

NAME	FORMULA	MOL. WT.
I Dimethylamino ethyl para-amino-benzoate hydrochloride	(p)$NH_2C_6H_4CO_2CH_2CH_2N(CH_3)_2$ HCl $C_{11}H_{17}O_2N_2Cl$	244.5
II Diethylamino ethyl para-amino-benzoate hydrochloride (Procaine)	(p)$NH_2C_6H_4CO_2CH_2CH_2N(C_2H_5)_2$ HCl $C_{13}H_{21}O_2N_2Cl$	272 5
III Di-n-propylamino ethyl para-amino-benzoate hydrochloride	(p)$NH_2C_6H_4CO_2CH_2CH_2N(C_3H_7(n))_2$.HCl $C_{15}H_{25}O_2N_2Cl$	300.5
IV Di-n-butylamino ethyl para-amino-benzoate hydrochloride	(p)$NH_2C_6H_4CO_2CH_2CH_2N(C_4H_9(n))_2$ HCl $C_{17}H_{29}O_2N_2Cl$	328.5
V Di-isopropylamino ethyl para-amino-benzoate hydrochloride	(p)$NH_2C_6H_4CO_2CH_2CH_2N(C_3H_7(iso))_2$.HCl $C_{15}H_{25}O_2N_2Cl$	300.5
VI Di-isobutylamino ethyl para-amino-benzoate hydrochloride	(p)$NH_2C_6H_4CO_2CH_2CH_2(C_4H_9(iso))_2$.HCl $C_{17}H_{29}O_2N_2Cl$	328.5
VII Di-secondary butylamino ethyl para-amino-benzoate hydrochloride	(p)$NH_2C_6H_4CO_2CH_2CH_2N(C_4H_9(sec))_2$.HCl $C_{17}H_{29}O_2N_2Cl$	328.5

Series B

NAME	FORMULA	MOL. WT.
I Diethylamino propyl para-amino-benzoate hydrochloride	$NH_2C_6H_4COO(CH_2)_3N.(C_2H_5)_2HCl$	286 6
II γDi-isopropylamino propyl para-amino-benzoate hydrochloride	$NH_2C_6H_4COO(CH_2)_3N$(iso propyl)$_2$HCl	314.6
III Di-normal propylamino propyl para-amino-benzoate hydrochloride	$NH_2C_6H_4COO(CH_2)_3N$.(N.propyl)$_2$HCl	314.6
IV Di-normal butylamino propyl para-amino-benzoate hydrochloride	$NH_2C_6H_4COO(CH_2)_3N$(N.butyl)$_2$HCl	342.7
V γDi-iso-secondary butylamino propyl para-amino-benzoate hydrochloride	$NH_2C_6H_4COO(CH_2)_3N$(sec. butyl)$_2$HCl	342.7
VI γDi-isobutylamino propyl para-amino-benzoate hydrochloride	$NH_2C_6H_4COO(CH_2)_3N$(iso butyl)$_2$HCl	342.7

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The corresponding B or propyl series was also furnished. This series differs from the A or ethyl series by being propyl para-amino-compounds instead of ethyl para-amino-compounds.

The local anesthetic effect of these drugs was determined by a number of methods, and while we do not consider any single method entirely satisfactory, they give information that cannot at present be procured by more desirable means. These methods have been previously used and justified by other investigators. The object of the investigation is the study of the nature of nerve impulse and anesthesia.

Anesthetic Value as Determined by Instilling Drug into Conjunctival Sac of a Rabbit's Eye

By S. J. Cohen, M.S., M.D., Chicago

METHOD

THE hair around the eye was clipped short to lessen the probability of touching or stimulating it during the experiment. The conjunctival sac was then filled with the anesthetic solution by retracting the lower lid, forming a pocket and filling it with the solution. This was allowed to remain in contact with the cornea for one minute. The excess of the anesthetic was then removed by allowing the eyelids to contract to their normal position. The point of a toothpick, wrapped in cotton, was applied to the cornea to test the winking reflex at one-half minute intervals.

An attempt was made to find a stimulus that could be stated in units (weight), but no satisfactory method could be devised. Since, however, the method used was accurate within a minute or two, we think it is quite sufficient.

The solutions used were of equimolecular concentration, and corresponded to 1 per cent procaine.

As a standard of comparison, the time required for the induction of anesthesia, likewise its duration, was determined in the following eight dilutions of cocaine: 4 per cent, 1 per cent, ¾ per cent, ½ per cent, ¼ per cent, ⅛ per cent, 1/16 per cent, 1/32 per cent.

These results are rather interesting. The onset of anesthesia of the various dilutions of cocaine was all within the time of application of the drug, i.e., one minute. The duration of anesthesia, with solutions up to

Table I
The Average Results with Each of the Dilutions

Cocaine HCl	Duration of Anesthesia (in Minutes)
4 per cent	48.00
1 " "	42.00
¾ " "	38.00
½ " "	23.30
¼ " "	17.00
⅛ " "	10.00
1/16 " "	5.00 (incomplete anesthesia)
1/32 " "	No anesthesia produced

1 per cent, varies directly with the concentration—the higher the concentration, the longer the duration of anesthesia and vice versa. However, there is no prolongation of anesthesia after a concentration of about 2 per cent. The only difference observed with the stronger solutions is the toxic effect of the drug on the cornea, as shown by drying, pitting, corrosion, etc. In concentrations lower than $\frac{1}{10}$ per cent there is practically no anesthesia induced so far as the rabbit's eye is concerned. Since the time of onset of anesthesia is the same with all these concentrations, it is clear that the time of onset cannot be used as a criterion of the anesthetic power of the solution.

Studies were then made with the ethyl-para-amino-benzoate compounds. The method used was as described above. One per cent novocaine was used as the standard (No. 2) and the other solutions were used in a corresponding molecular weight solution.

TABLE II

THE AVERAGE RESULTS OF THE NORMAL COMPOUNDS

DRUG	MOL. WT.	DURATION OF ANESTHESIA (IN MINUTES)
I	244.5	No anesthesia
II	272	14.00
III	300.5	16.00
IV	328.5	30.00

The isocompounds also act in relation to their molecular weight, but they are more toxic and irritant than the corresponding normal compounds. Table III shows the serial action.

TABLE III

ETHYL SERIES

DRUG	MOL. WT.	DURATION (IN MINUTES)
V	300.5	23
VI	328.5	45 (irritant)
VII	328.5	46

Studies were then made on a second series of anesthetics of the propyl-para-amino-benzoate compounds—six in all. Table IV gives the average results.

TABLE IV

DRUG	MOL. WT.	DURATION OF ANETHESIA (IN MINUTES)
I	286.6	26
III	314.6	35
IV	342.7	42

TABLE V

PROPYL SERIES

DRUG	MOL. WT.	DURATION (IN MINUTES)
II	314.6	60
VI	342.7	Too irritant for use
V	342.7	63

The results of these experiments are practically in the same order as the first series of drugs, except that in the propyl series the molecular weight is greater, and the increase in the duration time of anesthesia is correspondingly longer. When the anesthetic action of the para-amino compounds is

compared with the action of cocaine hydrochloride, their anesthetic efficiency on the rabbit's eye, arranged in terms of cocaine, is as shown in Table VI.

TABLE VI
COCAINE EQUIVALENTS
ETHYL SERIES

DRUG	
I	No anesthetic power
II	3/16% cocaine
III	1/4%
IV	5/8%
V	7/16%
VI	2%+
VII	2%+
PROPYL SERIES	
I	1/2% cocaine
II	4%+
III	3/4%
IV	1%
V	4%+
VI	Very irritant

From these results the occlusion may be drawn that the anesthetic efficiency of this homologous series of preparations in the rabbit's eye is proportional to the increase in the length of the normal chain, and to the increase in the molecular weight of the drug. Since these solutions were compared in molecular equivalents, and the molecular weights range from 244.5 to 342.7, there is a correction which must be made if a strict comparison of percentage solutions is desired. The molecular weight of procaine is 272.5. This was made in 1 per cent solution. If, therefore, one wishes the actual percentage comparison figures, it may be computed as follows:

272.5 : net weight of unknown :: 1 : x.

The molecular weight of cocaine hydrochloride is 339.65, so that no decidedly unfair ratio is obtained by assuming that molecular and percentage solutions are, for practical purposes, the same.

The toxicity of all these drugs, except No. IV in A series, is less than cocaine, and but slightly greater than procaine. They may, therefore, be used as local anesthetics in such regions as the eye. In the A series, Nos. V and VII seem especially worthy of consideration. No. VI, because of its caustic action, should not be used. In the propyl series, Nos. II and V deserve especial consideration. No. VI is too irritant for use.

THE STANDARDIZATION OF LOCAL ANESTHETICS BY THEIR APPLICATION TO THE SCIATIC NERVE TRUNK OF A FROG*

BY L. W. SCHULZ, D.D.S., CHICAGO

AFTER considerable experimentation, we have selected the following method: Frogs of approximately the same weight (25 grams) were decapitated just posterior to the optic lobes and before use were allowed to

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rest for at least two hours to recover from the shock. This period of rest is necessary to avoid marked difference in the response to the stimulus.

The frog was fastened to a cork plate by means of pins passing through the lower jaw and toes of the hind legs. A constant minimal stimulus from an inductorium was then applied to the webbed portion of the foot by means of a small electrode. This gave a positive contraction of the muscles anterior to the anesthetized area on that side of the body. This reaction was found positive in all the frogs tested.

The skin was now opened in a straight line for about two centimeters on the lower third of the dorsal surface of the thigh. This incision was made in the groove between the muscles. The fascia was then carefully separated by blunt dissection and the muscles pushed aside, permitting a full exposure of the sciatic nerve. Small hooks mounted in the ends of glass rods make very good instruments for this purpose. The muscles, skin, and fascia were retracted by small retractors made from safety pins, and the nerve exposed so that one centimeter of it could be immersed in the anesthetic. This leaves the nerve exposed in the bottom of a small diamond-shaped trough or well formed by the muscles, with one centimeter of the nerve anesthetized

The anesthetic (procaine 1 per cent in physiologic salt solution) is now put into this well by means of a dropper. The foot of the frog is then stimulated every ten seconds after the anesthetic has been in contact with the nerve for three minutes, when there is no response (contraction) of muscles anterior to the anesthetized area on this side. This is the "sensory end point" and its average time is about *five minutes* for anesthesia. The "motor end point" is determined by stimulating the nerve trunk direct, central to the anesthetic, and getting a contraction of the gastrocnemius muscle. The average time for the motor paralysis was found to be 14.1 minutes. This stimulation of the nerve direct is not desirable, but any other form of stimulation central to the anesthetic gives a marked contraction of other muscles higher up in the leg and trunk, and this obscures the end point.

DETERMINATION OF COCAINE VALUES

The time for complete anesthesia with cocaine solutions was determined by the method already described. The results are:

COCAINE	AVERAGE TIME FOR ANESTHESIA
4%	0′ - 37″
2%	1′ - 39″
1%	2′ - 4″
½%	2′ - 30″
¼%	4′ - 31″
⅛%	8′ - 5″

From ten to sixteen trials were made for each average reading in order to reduce the error. The results were then plotted on graph paper and the results obtained from the series of para-amino compounds compared with it.

DETERMINATION OF PARA-AMINO BENZOATE VALUES

The solutions used were of equimolecular concentration, corresponding to one per cent procaine. The A series of drugs, seven in number, produced sensory anesthesia in the following time:

A	I	Average	time	13′ – 57″
A	II	"	"	5′ – 30″
A	III	"	"	1′ – 42″
A	IV	"	"	1′ – 30″
A	V	"	"	1′ – 5″
A	VI	"	"	3′ – 55″
A	VII	"	"	2′ – 0″

These average times also were obtained from ten to sixteen experiments with each drug.

These solutions compare with cocaine as follows:

A	I	=	1/40%	cocaine	sol.
A	II	=	1/6%	"	"
A	III	=	1.7%	"	"
A	IV	=	2%	"	"
A	V	=	3%	"	"
A	VI	=	1/4+%	"	"
A	VII	=	2%	"	"

These results indicate the onset of complete anesthesia only. If the anesthetic is applied topically, its effect will not last as long as it will if the nerve is blocked by injecting the solution into the tissues. The time of onset of anesthesia is governed by the strength of the anesthetic, and the duration is governed by the power of absorption, destruction, or neutralization of the agent by the animal.

THE STANDARDIZATION OF THE B SERIES OF PARA-AMINO COMPOUNDS

This series of anesthetics was standardized the same as the previous groups, viz., on the sciatic nerve trunk of frogs. The inductorium was set at 8 cm. with a straight secondary coil. Stimulus was applied to the skin on the foot of the frog every five or ten seconds after the approximate time for anesthesia was determined. The temperature of the room was about 23° C. and the frogs were kept moist for about two hours after decapitation.

The results for the B series of local anesthetics were as follows:

B	I	Average	time	of	sensory	anesthesia	2′ – 32″
B	II	"	"	"	"	"	3′ – 0″
B	III	"	"	"	"	"	2′ – 8″
B	IV	"	"	"	"	"	1′ – 15″
B	V	"	"	"	"	"	1′ – 53″
B	VI	"	"	"	"	"	2′ – 20″

These solutions compare with the following strength of cocaine according to the curve plotted in previous experimentation, as follows:

B	I	=	approximately	a 0.5%	cocaine	solution.
B	II	=	"	a 0.4%	"	"
B	III	=	"	a 0.9%	"	"
B	IV	=	"	a 2.7%	"	"
B	V	=	"	a 1.3%	"	"
B	VI	=	"	a 0.7%	"	"

This method of comparison and standardization should be carried out at different intervals of a few days to a few weeks or months to see if these drugs will have the same anesthetic effect after aging.

Judged from the results of this method, Nos. III, IV, V, and VII of the ethyl series are very efficient local anesthetics, also Nos. IV and V of the propyl series.

Table I shows the results obtained on twenty frogs with one per cent procaine:

TABLE I

FROGS	TIME FOR SENSORY ANESTHESIA		TIME FOR MOTOR PARALYSIS	
	Right Leg	Left Leg	Right Leg	Left Leg
1	5½	5¾	16½	12½
2	5½	5¼	11½	14
3	5½	6	13¼	15½
4	4½	5	11	13½
5	4	5¾	15¼	16
6	5	4½	14½	15
7	6	5½	15	16¼
8	5¼	4½	11¾	16¾
9	4¾	5¼	16½	25¼?
10	5¼	6	13	14¼
11	5½	5	15	16¼
12	5	5¾	13	13½
13	5¾	5¾	16½	17
14	4½	4¼	15¾	16½
15	4	4½	15½	16
16	4	3¾	15	16
17	4¼	4	14½	15
18	5	4¾	15	14
19	4½	5	14	15¼
20	4¾	4½	15½	16
	Sensory Average 5 Min.		Motor Average 14.1 Min.	

DISCUSSION

The method outlined permits the study of local anesthetics in situ. Trauma and shock are reduced to a minimum. The nerve is immersed in the anesthetic in a manner which closely approximates the application of local anesthetics in actual practice. It permits the study of both sensory and motor paralysis and gives a valuable method of evaluating local anesthetics. A possible objection that may be brought against this method is that the muscles are also bathed by the anesthetic. This is unimportant because after sensory and motor nerves are both paralyzed, the muscles still react to direct stimulation. There is therefore no reason to suspect that the action on muscle is important or that the strength of the anesthetic bathing the nerve is changed to any important extent.

From the experiments carried out as outlined, the following conclusions may be drawn:

I. Absorption of the anesthetic has no apparent effect.

II. Sensory paralysis occurs before motor paralysis.

III. The anesthetic and paralytic power of a chemical series increases with the increase of molecular weight of the substituting alkali radicles. Ratio of the time of sensory to motor paralysis by this method is about 1 to 3.

The Relation Between the Time for Paralysis of the Sensory and Motor Fibers of a Nerve by Various Local Anesthetics as Determined by Their Action on the Sciatic Nerve of the Frog

By O. V. Pawlisch, B.S., Chicago

Practically all methods of determining the time of paralysis in the frog's sciatic nerve by local anesthetics refer to motor paralysis. In the present investigation I have endeavored to find the relation between the time of sensory and motor paralysis, and whether this relation varies with different anesthetics.

Ostlund, Hodges, and Dawson[1] report experiments showing the effect of heat block on the sciatic nerve of the frog when the nerve was exposed and carried transversely through a rubber tube containing heated saline solution. They found that as the temperature was raised the sensory fibers were paralyzed first, but they have no definite time ratio between sensory and motor paralysis. Hafemann[2] had previously performed similar experiments with corresponding results.

The basis for the greater susceptibility of the sensory fibers is not known. The idea that it is due to a superficial situation of the sensory fibers is not sustained by the work of Kraus and Ingham,[3] and our discovery that not all the local anesthetics (notably apothesine) paralyze the sensory first confirms this observation. There remains then the possibility of a difference in chemical composition. It may be due to a difference in the lipoid solubility coefficient or in a lipoid difference in the composition of sensory and motor nerves, as discussed by R. Gottlieb[4] in his article "Pharmacological Investigation Concerning the Isomers of Cocaine."

Our work was done on frogs, but we employed several methods to arrive at our conclusions, and each method with its results is given below. In all cases the animals were decapitated at the medulla and pithed backward to the foramen magnum. The best results were obtained with frogs which were allowed to rest an hour or two after the operation before tests were made.

Method 1.—The thigh muscles were cut away, leaving only the sciatic nerve connecting the leg to the body. The frog was placed on a paraffin block and the nerve was led through a raised cistern, 0.5 cm. in diameter, filled with the anesthetic. Stimulation was produced by a constant definite faradic current, with the secondary coil at 3. The end points were as follows: For sensory—stimulation of the nerve trunk peripheral to the block and production of crossed reflex or twitching of body. For motor—stimulation of nerve trunk central to block, and production of contraction of calf or foot of the corresponding leg.

This method has some disadvantages, notably the possible injury to the nerve by direct stimulation, exposure to the air, and the fact that end points

TABLE I

RESULTS BY METHOD 1

NO. OF TRIALS	ANESTHETIC	SENSORY PARALYSIS OCCURRED IN	MOTOR PARALYSIS AFTER	SENSORY MOTOR RATIO
13	No anesthetic	17′ 9″	40′ 35″	42%
11	0.25% Cocaine	11′ 43″	22′ 7″	53%
12	0.50% "	4′ 58″	11′ 23″	43%
14	1% "	1′ 40″	3′ 17″	50%

were faint and in some frogs absent. A significant result is that the sensory paralysis occurs first, even without the use of an anesthetic, the nerve being merely exposed to the air. It is interesting to note that some of these experiments were performed during very hot weather in July, and some during cooler weather in October. During the hot weather the S. M. ratio was 38 per cent and during the cooler weather 51 per cent. This seems to be in accord with the work of Ostlund, Hodges, and Dawson on heat paralysis.

Method 2.—A pocket was made in the thigh by spreading the muscles around the sciatic and holding them apart with a clip, the nerve being exposed for about one centimeter of its length. The frog was placed on a cork plate and pinned down, the pocket being filled with the anesthetic. End points: Sensory—stimulation of the skin over the toe with faradic current and production of contraction or twitch of body of other leg. Motor—stimulation of skin over lumbar cords and contraction of calf or foot.

TABLE II

RESULTS BY METHOD 2

NO. OF TRIALS	ANESTHETIC	SENSORY PARALYSIS	MOTOR PARALYSIS	SENSORY MOTOR RATIO
6	0.50% Cocaine	2′ 28″	11′ 30″	21.7%
6	1% "	1′ 54″	6′ 20″	30 %

This method has the advantage of avoiding the exposure and injury to the nerve which occurred in Method 1. It has the same disadvantage of no sharp sensory end point, and also that the stimulation over the lumbar cords was not exactly analogous with the sensory stimulation of the skin of the foot. This we believe accounts for the prolonged motor paralysis time.

Method 3.—Frogs were sensitized by a dose of strychnine just sufficient to produce a crossed reflex without severe tetanic convulsions (0.10 to 0.15 c.c. of a 0.01 per cent solution). A thigh pocket was made as in Method 2, but arranged so that when the frogs were suspended by the Türck Method the anesthetic would not run out. End point: Sensory—toe of blocked leg

TABLE III

RESULTS BY METHOD 3

NO. OF TRIALS	ANESTHETIC	SENSORY PARALYSIS	MOTOR PARALYSIS	SENSORY MOTOR RATIO
4	1% Cocaine	2′ 12″	5′	44%

placed in 0.5 per cent H_2SO_4, and withdrawal of both legs. Motor—immersion of toe of unblocked leg, and the raising of both legs.

The results corroborate closely those of Method 1, but the method is attended with difficulty. An advantage is that the stimulus may easily be kept constant.

Method 4.—Frogs were sensitized with strychnine as in Method 3, but placed on cork plate as in Method 2. End points: Sensory—faradic stimulation of skin of toe in blocked leg and production of contraction in opposite leg. Motor—faradic stimulation of skin of toe of unblocked leg, and production of contraction in blocked leg.

TABLE IV

RESULTS BY METHOD 4

NO. OF TRIALS	ANESTHETIC	SENSORY PARALYSIS	MOTOR PARALYSIS	SENSORY MOTOR RATIO
5	1% Cocaine	3′ 8″	6′ 12″	50.5%
4	1% B-Eucaine	7′ 11″	13′ 41″	52%
6	1% Butyn	5′ 34″	6′ 25″	87%
5	1% Apothesine	12′	11′	110%
5	1% Novocaine	7′ 45″	14′ 30″	53%
2	A-1	Not anesthetic		
4	A-2	8′ 37″	13′ 22″	64%
5	A-3	3′ 09″	3′ 48″	82%
6	A-4	3′ 15″	6′ 05″	53%
5	A-5	5′	6′ 54″	72%
4	A-6	6′ 37″	6′ 30″	101%
4	A-7	7′ 45″	8′ 45″	88%
4	B-1	12′ -7″	14′ 07″	85%
4	B-2	8′ 30″	9′ 52″	86%
6	B-3	5′ 50″	6′ 35″	88%
5	B-4	5′ 24″	6′	90%
5	B-5	9′ 06″	11′ 06″	82%
4	B-6	8′ 37″	9′ 45″	88%

CONCLUSIONS

1. That when a nerve is exposed to the action of various local anesthetics, the sensory and motor fibers are not paralyzed at the same time.

2. That for each local anesthetic there is a fairly definite ratio between the time for motor and the time for sensory paralysis.

3. (A) That cocaine, B-eucaine, butyn and novocaine paralyze the sensory fibers first; (B) That apothesine paralyzes the motor fibers first. (Details in Table IV.)

4. That this selective action is not due to a superficial location of the sensory fibers.

5. That under exposure to heat and drying, the sensory fibers lose their power of conduction before the motor fibers as they do under anesthetics.

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USE OF A SUCTION APPARATUS FOR BLEEDING RABBITS*

By C. M. Anderson, M.D., C.P.H., Toronto, Canada

THE three methods most commonly employed in bleeding rabbits which have been successfully immunized against a specific antigenic substance are:

(1) Cutting the marginal ear vein.

(2) Severing the vessels of the neck.

(3) Aspiration from the heart by the use of a syringe.

The third method is probably the method of choice with most laboratory workers on account of the ease with which the blood may be obtained in a sterile condition. It is, however, sometimes extremely difficult to obtain more

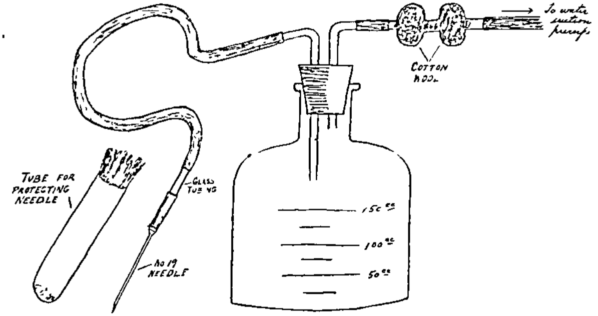

Fig. 1.

than 40 or 50 c.c. of blood by this method. A syringe with a capacity of 30 c.c. is about as large as can be conveniently handled in bleeding rabbits from the heart. When the syringe becomes filled, the operator must either withdraw the needle from the heart or disconnect the needle from the syringe and leave the needle inserted into the heart while the contents of the syringe are transferred to a sterile centrifuge tube. I have frequently seen experienced workers produce a satisfactory titer in a rabbit but fail to obtain more than 30 to 40 c.c. of blood.

With the apparatus described and illustrated, one can practically exsanguinate the animal with one puncture of the heart. Yields of from 90 to 100 c.c. are easily obtainable.

We make up about six of the above apparatus at one time. These are wrapped in cotton towelling and sterilized in the autoclave for one-half hour

*From the Public Health Laboratories, Department of Health of Ontario.
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at 15 pounds. Those not used in a month's time are again sterilized. By this practice we have the apparatus constantly available.

It is essential to use a good sharp needle entirely free from rust or other partial obstructions. The only difficulty encountered with the apparatus in our laboratory has been due to lack of attention to this detail.

The rabbit is etherized, the hair over the cardiac area clipped short, and the skin painted with tincture of iodine. The apparatus is attached to a water suction pump and the operator inserts the needle into the heart over the point of the cardiac impulse. When the heart has been entered, the blood will be observed to flow up through the glass window into the collecting flask. The needle is held in position until the last drop of blood is obtained.

Upon completion of the bleeding, the blood is allowed to stand one hour at room temperature for clotting to take place. The rubber stopper and connections are then replaced by a sterile cotton plug and the flask is placed in the refrigerator overnight to allow the serum to separate completely. The following morning the serum is removed from the clot. Usually only a small portion of serum will require centrifuging. The serum is then mixed with an equal volume of C.P. glycerin and dispensed into vials of 5 c.c. capacity.

The apparatus may also be used for obtaining sterile defibrinated rabbit blood for enrichment of media. When the apparatus is used for this purpose, glass beads are placed in the flask and while the bleeding is proceeding, the flask is gently shaken to prevent coagulation. The rabbit need not be bled out unless desired. The defibrinated blood is stored in the cold room and used as required to make blood agar slants or plates.

The apparatus has been in use for over a year with consistently good results.

IMPROVED FORMS OF SEVERAL COMMON LABORATORY APPLIANCES*

By J. P. Quigley and W. C. Heath, Augusta, Georgia

SEVERAL pieces of apparatus used in the biologic sciences have recently been developed in this laboratory. They appear to be superior to those formerly used and are so easily made that it seems advisable to publish a description of them.

Frog Heart Four-Way Perfusion Tube.—This consists of a block of brass with holes drilled at right angles to each other. Brass tubes are screwed into the holes and the tubes connected to reservoirs containing the perfusion fluid. Fig. 1 (C). The apparatus is easily cleaned, does not get out of order, is very strong and holds a small amount of fluid.

Signal Magnet.—This apparatus is made from an electric door bell by removing the frame containing the magnets, removing the striker ball and

*From the Department of Physiology and Pharmacology, Medical Department University of Georgia, Augusta, Georgia.

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also one of the magnets, see Fig. 1 (B). The wires are so arranged that the current does not flow through the interrupter but instead makes a permanent magnet. This signal is decidedly more durable and has a stronger movement than those now on the market.

Drop Recorder.—The foundation of the recorder is a Harvard heart lever. A cup made of cork and paper coated with shellac is attached to the lever. The cup has perforations around the edge to permit the escape of the fluid.

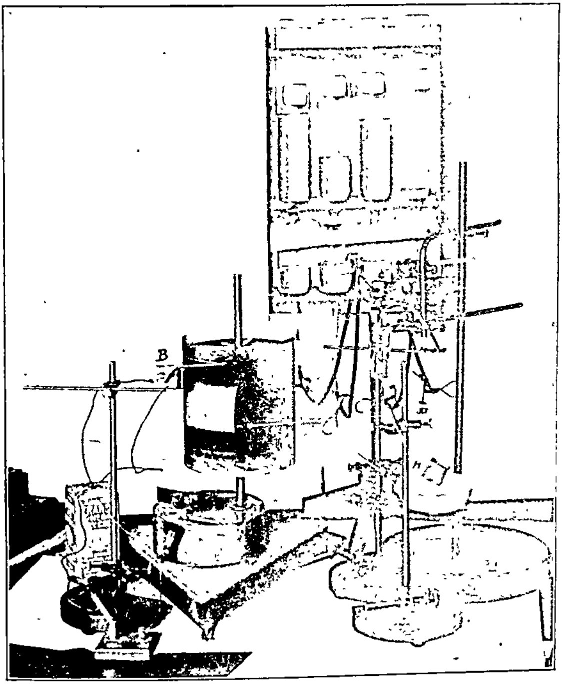

Fig. 1.—One of the possible uses of (*A*) drop recorder, (*B*) signal magnet, (*C*) 4-way heart perfusion tube.

A piece of wire fastened to the bearing support of the heart lever projects for about an inch towards the writing end of the lever. A small piece of dental dam connects the lever and this piece of wire, see Fig. 1 (A). This recorder is easily made, is inexpensive, and rarely requires attention.

Anesthesia Regulator.—For anesthetizing animals when a tracheal cannula is employed, this apparatus permits of an unusually accurate control of the rate at which the anesthetic is given and when the stage of surgical anesthesia is attained becomes almost automatic in its operation. This appa-

ratus is made from the strainer of a percolator and an automatic oil cup. A piece of sponge is placed inside the strainer and the apparatus mounted on a plate or block of wood, see Fig. 2. Ether or chloroform is placed in the

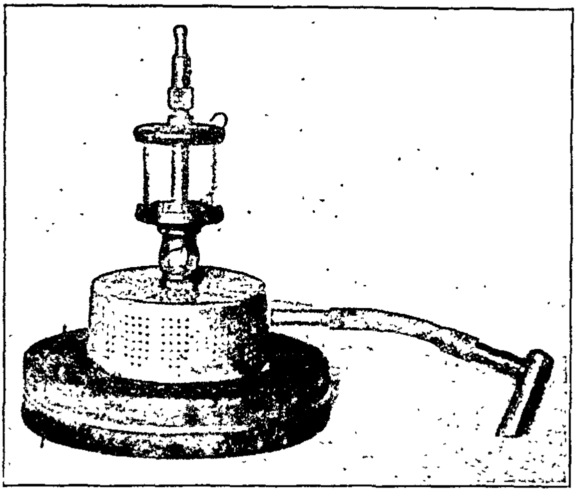

Fig. 2.—Anesthesia regulator.

glass cup and the rate of flow readily and carefully controlled by the adjustment screw and valve lever at the top of the cup.

We wish to express our appreciation to William Salant whose interest in this work has been of material assistance.

A SUBSTITUTE FOR THE RUBBER BULB FOR USE IN THE KOLLS AND ERLANGER BLOOD PRESSURE INSTRUMENTS*

BY LAWRENCE S. KUBIE, M.D., BALTIMORE, MD.

SATISFACTORY records can be obtained from either of these instruments only if one has a rubber membrane which is just sensitive enough, and neither too rigid nor too soft, to transmit the pulsations of the pressure system to the writing lever. The rubber bulbs which have always been used for this purpose are now very hard to obtain. They are no longer manufactured in this country; and large supplies cannot be imported because the fabric rapidly loses its elasticity. Therefore, we have devised a very simple and inexpensive substitute for these bulbs and have found it entirely successful. During the past winter so many inquiries have been received at this laboratory in regard to a possible source of the rubber bulbs, that it seems advisable to publish a diagram of this substitute.

The diagram is almost self-explanatory. Essentially the instrument con-

*From the Department of Physiology, The Johns Hopkins Medical School, Baltimore, Maryland.
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sists of a brass hemisphere instead of the glass bulb of the Kolls or Erlanger apparatus, and a rather large rubber tambour in the place of the rubber bulb. The hemisphere screws down over the tambour and when pressure is pumped into the cuff around the arm the rubber tambour is stretched until it nearly fills the cavity of the brass hemisphere. Its pulsations within the hemisphere then correspond to the pulsations of the rubber bulb within the glass bulb of the instrument in its usual form. We merely substitute the pulsation of a hemisphere of rubber within a hemisphere of brass for those of a sphere of rubber within a sphere of glass. The hemisphere can, of course, be made of glass, and can be sealed to the base plate with sealing wax. The rubber

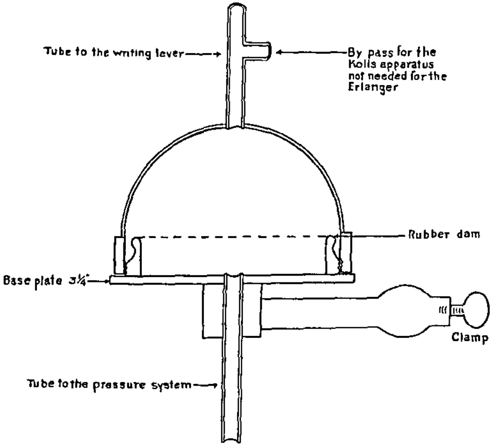

Fig. 1

dam that is used in the tambour will vary with the work to be done; any weight and tension can be used. For classroom work we have found that a double thickness of fairly heavy dam was more sensitive than the rubber bulbs and had less tendency to fling, giving far more consistent records than had been obtained with any rubber bulbs which we could secure.

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CONCERNING THE INOCULATION OF ANIMALS WITH SOLID INOCULUM*

By G. H. Hansmann, M.D., Iowa City, Iowa

TISSUE inoculations into animals for diagnostic purposes are frequently attended with difficulties, and the results are not always reliable in that they do not conform with the histologic findings. The greatest difficulty is encountered in those cases where the lesion is one attended with little necrosis or exudation and much productive reaction. We have devised the following method which is simple and has proved satisfactory in our hands.

METHOD

1. Materials needed are fine silkworm-gut, scissors, long cutting needle and tissue forceps.
2. The tissue is attached to the silkworm-gut which is in turn threaded into a long cutting needle.
3. Incision is made in the abdominal wall with the scissors.
4. The needle is entered in the abdominal incision and drawn out in the groin drawing the tissue against the abdominal wound.
5. The lower flap of the wound is retracted and with steady traction the tissue slips into the groin.
6. The remaining silkworm-gut may then be used for a stitch or two in the wound of the abdominal wall.

The ease with which this procedure is accomplished, the lack of objection shown by the animal, the small amount of tissue injury and the rapidity with which healing takes place are all remarkable. The pain shown by the animal is much less than that resulting from the building of pockets in his anatomy in which to place the tissue not mentioning the insecurity of the collodion-cotton pocket seals and what happens to the tissue when the pockets are inverted. The tissue inoculated by the above method is held firmly in place and the tract taken by the inoculated tissue seals immediately and in from two to three days is healed quite firmly.

The tissue applicable to this type of inoculation is synovial tissue, bone and quiescent tuberculous lesions in which much fibroblastic reaction is present. It is in these types of lesions that the discrepancy between histologic and animal inoculation results is greatest and also where a positive animal is of greatest value.

We believe that the procedure outlined is an improvement on methods commonly employed in the inoculation of human tissues into animals for diagnostic purposes.

*From the Laboratory of Pathology, University Hospital, Iowa City, Iowa.
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A SIMPLE DEVICE FOR HASTENING FILTRATION THROUGH BERKEFELD FILTERS*

By F. A. Stenbuck, M.S., New York City

THE filtration of slow filtering colloidal solutions through filters of the Berkefeld type may be expedited where both air pressure and vacuum are available by the use of a simple device illustrated in the diagram. A piece of brass pipe about seven inches long is threaded at both ends and pro-

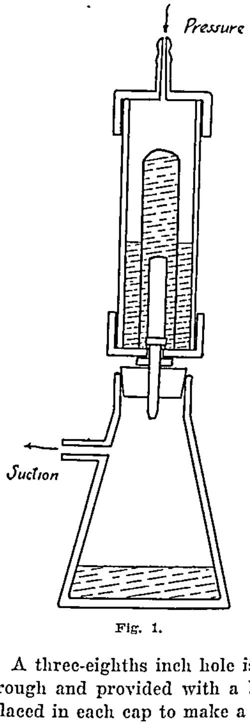

Fig. 1.

vided with brass caps. A three-eighths inch hole is drilled in one cap. The other cap is drilled through and provided with a brass stopcock nipple. A rubber ring gasket is placed in each cap to make a tight fit.

In using the device the stem of the filter candle is passed through the

*From the Pathological Laboratory, Mount Sinai Hospital, New York City.
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hole in the cap and tightened with the usual lock nut on the outside. The cap is screwed onto the pipe tightly and the solution to be filtered is placed in the pipe, as in the mantle of the usual filter. A test tube large enough to fit loosely over the filter candle is filled with the solution and inverted over the candle to insure the use of the whole candle surface while there is any liquid left in the mantle. The top cap is screwed on tight and connected to the source of compressed air. Both suction and pressure may then be used at the same time. Filtration may be further speeded up by opening the apparatus when the flow of liquid through the filter stops, and removing the film of slime from the surface of the candle with a small brush, such as a toothbrush.

In this way we have reduced very considerably the time necessary to filter solutions containing heavy suspensions of bacterial débris.

TO DRY AND CLEAN HYPODERMIC NEEDLES*

By Walter M. Brunet, M.D., Brooklyn, N. Y.

THE care of hypodermic needles is, at best, a disagreeable task and oftentimes after a hypodermic or other injection, the needle is laid aside to be cleaned and dried later. Usually when the job of cleaning the needle is reached it is found to be clogged up. Oxidation is the foe of all steel needles and this destructive power is due to the fact that needles are put away without being properly dried.

The simple inexpensive instrument here illustrated is for the purpose of drying and cleaning hypodermic needles.

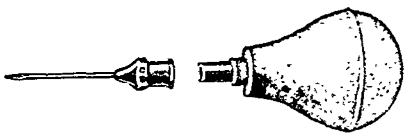

Fig. 1.

It consists of a B-D Asepto rubber bulb, ½ ounce capacity, into the neck of which is inserted a male Luer adapter. This adapter fits all Luer needles.

To use this instrument, the adapter is inserted into the hub of the needle and the bulb compressed several times. In this manner, the in and out action of the air through the needle will blow out the moisture and dry it thoroughly.

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EDITORIALS

Blood Platelets and Leucocytes in Tuberculosis

IN 1914, some students of tuberculosis at Colorado Springs reported the following observations:[1, 2, 3]

1. At an altitude of 6,000 feet, blood platelets are increased in the circulating blood of man and guinea pigs. The average of counts made on one hundred healthy young men at sea level (New York) was 302,000; at 6,000 feet (Colorado Springs) 340,000, an increase of about 12 per cent.

2. Blood platelets are consistently increased in tuberculosis of man and guinea pigs. The counts in healthy guinea pigs were found to average somewhat over 400,000; in tuberculous animals somewhat over 6,000,000. In man the findings were classified:

(Normal 340,000)

Early afebrile tuberculosis,	45 counts,	35 patients	— 368,000
Advanced quiescent tuberculosis,	50 "	40 "	— 412,000
Advanced active tuberculosis,	26 "	22 "	— 496,000

Quite recently Bannerman[4] has reported a similar series of observations made at Montana in Switzerland, at an altitude of 5,000 feet. His platelet count on normal individuals (number not stated) varied from 250,000 to 380,000, the average being 300,000. He does not report any counts on normal persons at sea level by his technic, which is different from the methods commonly used. He made counts on 65 cases of pulmonary tuberculosis, and found, as did the workers at Colorado Springs, "that the blood-plates are generally present in excessive numbers in active pulmonary tuberculosis, and that, broadly speaking, the more serious the clinical condition the greater the degree of thrombocytosis."

As to the application of these facts, Bannerman writes, "It appears that there exists an inverse relation between thrombocytosis and the subject's resistance to the disease; it is suggested that the plate count may afford a means of measuring that somewhat indefinite entity, which is the basis of prognosis."

The earlier investigators of the subject considered this possibility, but it soon became apparent that in tuberculosis those classes of patients who showed increase of platelets showed an approximately parallel increase of polymorphonuclear leucocytes; the platelet count was, therefore, deemed a superfluous addition to the routine examination of blood in this disease, and was given up.

It may be added that of all blood examinations, morphologic, serologic, and clinical, the one which is of the greatest use in the prognosis of tuberculosis is the differential leucocyte count. The facts, noted by Ullom and Craig[5] about twenty years ago, and since amply confirmed by many others, may be epitomized thus: The more polymorphonuclears, the worse the prognosis; the more lymphocytes, the better the prognosis.

In a paper[6] published in 1923, the results of over 2000 counts on some 400 tuberculous patients at Colorado Springs were analyzed and tabulated thus:

	"Lymphocyte index"
I. 100 normal adults	43
II. 130 arrested or approximately cured cases	45
III. 120 cases improving	37
IV. 100 cases failing	24
V. 40 cases progressing to death	16

The "lymphocyte index" comprises the aggregate percentages of large and small lymphocytes, large mononuclears, and "transitionals." The so-called transitional cell is, of course, a form of the large mononuclear. In the light of our studies and those of others it would perhaps be better to omit these cells from the index, since they do not, like the lymphocytes, vary directly with the favorableness of the patient's condition. Our findings were as follows:

	Large mononuclears Per cent	Large mononuclears No. per cu. mm.
Normals	8	624
Arrested and cured cases	8	608
Far advanced active cases	6	840

McJunkin and Charlton,[7] in 1919, reported that these cells may be increased to as much as 10 per cent in advanced tuberculosis of guinea pigs, while in the normal animals they averaged 1.5 per cent. Further observations on this point have agreed with these, and it appears that in guinea pigs a high percentage of such cells indicates rapidly progressing disease, while in them, as in man, a high lymphocyte count indicates relatively effective resistance.

Like the erythrocytes and the platelets, the lymphocytes are increased at high altitudes. This fact, first reported in 1909,[8, 9] has been widely substantiated.[10, 11, 12, 13]

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—*G. B. W. and C. T. R.*

The "Face Value" of the Wassermann Report

LIKE all innovations in medicine the Wassermann test, so-called, has undergone many vicissitudes. At first heralded with acclaim, later criticized and even reviled in some quarters, it is only within recent times that this procedure has approached a proper evaluation, and, strange to say, the establishment of the complement-fixation test in syphilis in its proper relation to the diagnosis and treatment of the disease has been as much the result of the efforts and teaching of the serologist as of the strictures, comments, and criticisms of the clinician.

Despite the gradual appreciation of the true status of the complement-fixation test as only a single symptom, a phase of syphilis; as only one part of what should be an extensive, intensive, and exhaustive examination at times embracing many specialized forms of investigation; despite the emphasis properly laid upon the fact that the most important and vital feature of the Wassermann report should be its interpretation, serologists are still occasionally exhorted to improve their methods of examinations so that "reports may with safety be accepted at their face value."

Reduced to its ultimate significance this is really a demand that the complement-fixation reaction in syphilis be made infallible, that reports bear the significance and authority attributed to the Delphian oracle, that the diagnosis of syphilis be stripped of all complexities and difficulties other than those attendant upon the collection of a specimen of blood. The treatment would consist solely of the attainment of serologic negatives.

This, of course, is impossible of achievement nor will there ever be any method of laboratory procedure devised in connection with the diagnosis and treatment of syphilis which will relieve the practitioner of the necessity for the careful and minute study of each individual case.

It is unfortunate that the conception of biologic specificity upon which the original Wassermann test was founded still exerts a subconscious influence.

The first blind reliance upon a positive reaction as irrefutable evidence of syphilis was followed, as was to be expected, by an equal degree of belief in a multiplicity of conditions capable of giving a false positive reaction and it is only within very recent times that the high degree of relative specificity of the test has been revealed by the painstaking investigations of numerous workers.

Refinements of technic have added much to the delicacy of the test so that the reacting substances may be detected and even measured when present in remarkably small amounts. The reagents and manipulative factors of the technic have been modified and refined to such an extent that the reliability of a positive reaction has been tremendously increased.

Much has been done, therefore, to enhance the "face value" of a positive report—but nothing appears possible of early achievement which will warrant the hope of infallibility for the Wassermann report in general.

It must always be remembered that an estimate of the reliability of a report is inseparably associated with an understanding of the reliability and delicacy of the technic whereby it has been attained. No two reports may safely be compared without a coincident comparison of the merits of the technic or methods involved.

The "face value" of a negative report remains simply this: it is a statement of failure to detect in the serum examined at the time of examination any bodies reacting with a syphilitic "antigen." It applies to the conditions obtaining only at the time of examination; it bears no relation to past conditions and holds no guarantee for the future. Its proper evaluation is entirely dependent upon the proper summing up, and the individual and collective evaluation of all the circumstances pertinent to the particular case.[1]

The true explanation of the complement-fixation test in syphilis remains to be elucidated. In so far as is known it is the result of an interaction between the tissues and the invading spirochetae. Obviously, therefore, the production of reacting bodies—best spoken of collectively as reagin—is directly dependent upon: (a) the activity of the spirochetae and their effect upon the tissues; and, (b) the ability of the tissues to react.

The detection of reagin, when produced, is directly dependent upon: (a) the amount in which it is produced, and (b) the delicacy of the method in use.

Consideration of these basic principles makes clear the reason for the occurrence of negative reactions in syphilis. They are due, either to a

[1]Kilduffe, R. A.: The Status of the Negative Wassermann, Jour. Am. Med. Assn., Dec. 30, 1922, lxxix, 2215.

quiescent state of the spirochetae in which their effects upon the tissues are reduced to a minimum, to a lack of ability on the part of the individual to react, or to a failure of the individual to produce reagin in amounts capable of detection by the method of complement fixation in use.

Certainly it is true that in undoubted cases of syphilis negative reactions occur and, what is not readily susceptible of explanation, such negative reactions may occur in patients exhibiting clinical evidence of active lesions and may follow within a relatively short time a previous positive reaction.

Such instances are common to the experience of both serologist and clinician and have been reported, though it must be said that the possibility of their occurrence is better appreciated by the serologist

Under these circumstances, therefore, the acceptance of a Wassermann report at its "face value" indicates a lack of understanding of the factors influencing the occurrence and detection of the reaction, and an equally serious lack of appreciation of the essential necessity for a careful study of each case upon its individual merits.

By methods in ordinary use, except that described by Kolmer, a positive reaction may be obtained in pregnancy in the absence of syphilis, especially when the cord blood is tested, in the febrile stage of pneumonia, in leprosy, and in yaws. The only nonluetic condition so far reported as giving a false positive reaction with Kolmer's quantitative technic is yaws.

By all the methods in common use, including Kolmer's, negative reactions may be obtained which do not represent the absence of syphilis, though with the latter technic the frequency of such false negative reactions is reduced to a minimum.

The "face value" of the Wassermann report, therefore, is simply that of a single, isolated examination. Its true value depends upon its correlation with and interpretation in the light of all the other findings: clinical, historical, and laboratory, of the individual case at hand.

—*R. A. K.*

The Periodic Health Examination

THE principles of public health were inaugurated in their crudest form long before the dawn of civilization, when savages, fearing the demons of disease, segregated and shunned the sick. The ancient Mosaic laws were in many respects models of public health and excellent applications of the principles of preventive medicine. Indeed, many applied directly to *individual* health and hygiene. Relics thereof still are seen in some portion of the modern church ritual. Mohammedans, for whom their prophet formulated similar great laws of personal hygiene, have maintained many in more nearly the original form. Thus, the Mohammedan obeisance which must be repeated at least thirty times daily, is an excellent exercise which in the Christian church has devolved into the simple genuflection. The cleansing of the entire body has but a relic in the modern dipping into holy water.

The recognition of the contagiousness of many diseases and the necessity

for protecting society against individual illnesses led to the establishment of public health measures which have developed until they, today, form the vast science of preventive medicine. While public health regulations benefit society as a whole, the individual also profits. Not infrequently *individual* preventive medicine has been applied in the past. Examples are antirabic treatment, the prophylactic administration of tetanus or diphtheria antitoxin, and vaccination against smallpox and typhoid fever.

The possibility of applying preventive medicine for the betterment of the individual was recognized decades ago. About 1870, Dobell, in England, suggested periodic examinations for life insurance companies. In 1900, Gould, of Philadelphia, read a paper before the American Medical Association urging the institution of health examinations by the family physician. Barés, in France, in 1902, discussed the possible value of periodic examination of healthy or apparently healthy persons.

In 1909, the Provident Life Insurance Company adopted the suggestion that medical examinations be made every five years. The Life Extension Institute of New York was organized in 1914 by a group of laymen and has since made over 250,000 examinations. Periodic examination of employees of the health department of New York City was begun in 1914. The annual examination of army officers was instituted in 1922. The National Health Council, a lay organization, desired to begin a campaign for yearly health examinations in 1922 but this was postponed for one year because the American Medical Association felt that physicians were not yet ready to cooperate in such a campaign. The National Health Council did institute its campaign in 1923, two years ago, with the slogan, "Have a Health Examination on Your Birthday." The American Medical Association endorsed the movement in 1922 and reiterated its endorsement in 1923 and 1924.

The Association first brought it to the attention of the medical profession in January, 1923, in an article by Dodson, and in May of the same year a concrete program for examination was presented in an article by Emerson. Since this time the idea has been put into practice by physicians in many of the larger centers and several articles dealing with the subject have appeared in the literature.

While individual preventive medicine deals chiefly with the prevention of metabolic, infectious, or other disturbances arising within the body, it also entails increasing of the bodily resistance against infection from without, either by promotion of general hygienic measures or by specific immunization. The aim is the establishment and maintenance of good health. As Tobey points out, good health is considerably more than mere disease dodging. "It is the ability so to live that the maximum benefit is derived from living. It is not only getting along without being ill, but it is that condition in which one is able to enjoy life. It is the perfect regular operation of the human machine so that there is the greatest productivity, utility, and potentiality."

In applying the principles of preventive medicine, we attempt to recognize chronic disease in its incipiency, and by clearing up predisposing causes such as focal infection, constipation, overeating, etc., to prevent or retard the progress

of its development. Indeed, in those cases in which disease has not yet made its appearance but in which certain faulty habits, errors of diet, etc., clearly predispose, we may point the way to right methods of living so that even the beginnings of disease may better be avoided.

The method of the health examination is essentially that used in the presence of obvious disease but the examiner's attitude is altered in that he must recognize the significance of minor abnormalities, determine as to their relative importance, and realize their potentiality for subsequent evil.

The examination must be most painstaking and comprehensive, not only for the gaining of the patient's confidence, but so that we may be sure that the earliest pathologic changes have not been overlooked

With the increasing complexity of modern civilization and its great dynamic urge, problems of individual health have arisen which were unknown to the ancient physician, practicing on a more nearly vegetative race. It is a weak point in our practice that while aiding the patient with outspoken disease, we are often unable to relieve the below-par individual, who knows that all is not well within his economy, but in whom no definite organic abnormality can be discovered. It is within this enormous group that the cultist, the Christian Scientist, the osteopath, the chiropractor, the Abramite, makes such tremendous inroads. Their self-confidence, the "promise of cure" and the absence of serious progressive constitutional disease often combine to give remarkable symptomatic benefit, which, however, is often of short duration.

Indeed, many of these charlatans are more experienced in instilling principles of right living than is the busy practitioner who finds it less time-consuming to write an offhand prescription for some drug of doubtful potency. Modern pharmacology has cut the drugs of proved worth to a remarkably small number. The giving of drugs outside this small list may have some temporary benefit but since they do not remove the true cause of the patient's distress, the results are no more lasting than those obtained by the cultist. True, the wholesale use of drugs in the past makes it difficult to convince the patient that health may be acquired through hygienic reconstruction, often without adjunct medicinal therapy, and I consider it often thoroughly legitimate to prescribe tonic medication, but this is of little value unless accompanied by explicit and detailed instructions in personal hygiene, the clearing up of chronic infection, etc. "The physician of today should be not a dispenser of pills but a purveyor of positive health." (Bill.)

There exists a great group of degenerative and metabolic diseases which, once developed, remain resistant in greater or less degree to all therapeutic measures. These can be attacked only in their incipiency or in predisposed cases by personal preventive measures.

Who is best qualified to advise and supervise the patient? Today, it is the visiting nurse, the school nurse, the social service worker, the life insurance company, the institution organized and directed by laymen, the health chat in the daily press, the "home journal," and so on, which bring the message of hygiene to the individual.

Pioneer work is now being accomplished through "Hygiea." The one per-

son fully qualified to advise in the individual case, who alone is acquainted with the patient's physical make-up, his tendencies toward emotional reactions, his antagonisms, his proneness or not to morbid anxieties, and who has intimate knowledge of the patient's domestic environment, his habits, his familial tendencies, his hereditary predispositions is the family physician. The physician, so qualified, is the appropriate person to direct the patient regarding his health and his personal hygiene. It is he alone who is qualified through experience to know what to emphasize and what should be minimized. Individual health, like community health, is the province of the physician and other organizations, many of which are of undoubted intrinsic worth, must necessarily serve as adjuncts and work in collaboration with the directing physician.

However, until the physician realizes this, equips himself properly to perform the function of health adviser and asserts his prerogative, the present situation will continue. What is the present situation? The health promotion institute makes a more or less detailed examination, gives a fairly comprehensive and apparently satisfactory report to the patient, and then advises, if any abnormality is found, that he consult his own physician for specific instruction. As it now stands, then, the problem comes back to the physician, but it is to him that it should have been originally presented. On the other hand, the patient, after examination by physicians working for a lay organization, receives the report that all is well, loses contact with his own physician, and contents himself with an annual repetition of his health study. Often some intrinsic unadjusted problem, such as a problem of the home which is known only to the family physician, remains undiscovered and undiscussed and later may give rise to trouble.

Then there is another type of examination given by nonmedical organizations; annual or periodic urinalyses. It is surprising what a large group of successful business men submit themselves for this examination and upon receiving a negative urinalysis report, settle back thoroughly content in the knowledge that they have been pronounced sound in every respect. They have been paying from ten to twenty-five dollars annually for a procedure which alone is of doubtful worth.

The average duration of life has been increased from forty to fifty-eight years within the last seventy-five years. This increase has been accomplished almost entirely through reduction in infant mortality and deaths from contagious disease in the earlier years of life. It is confined almost entirely to age groups under thirty-five. Above forty-five, there are more deaths per thousand than there were twenty years ago. During the last century there has been practically no improvement in mortality among the aged. With existing knowledge we cannot hope to increase the normal life span much beyond the biblical limit of three score years and ten. As yet medical science is unable to prevent the degenerative changes associated with senescence.

However, with the knowledge which we already possess, properly applied, we can accomplish much in delaying degenerative changes, particularly of the heart, kidneys, and blood vessels. Dublin has constructed a hypothetical life table which expresses the best mortality we may hope for with our present

knowledge. In it the total expectation of life at birth is sixty-five years. This means an addition of eight years to the life span now prevailing in the United States. Of course, a great deal of the improvement will result from decreased mortality in the earlier years of life, but the factor of earlier recognition and preventive treatment of chronic, infectious, metabolic, malignant, and degenerative diseases, plays a very definite part.

About 30 per cent of accepted life insurance risks supposed to be practically normal, succumb to cardiovascular diseases.

Three conditions: Heart disease, Bright's disease and cerebral hemorrhage, curtail the present expectation of life by about four years. These deaths represent heavy loss to the community because they often involve people still in the prime of life and at the height of their usefulness.

The productive period of man rarely extends beyond the age of sixty-five. It is our hope that the average duration of life may be extended through this period of productivity, but we must bear in mind that the application of individual preventive medicine is not designed only to decrease the *death rate* but also, and particularly, to prevent disability in those who, while still living, are made inefficient and unhappy by disease.

The extent of unrecognized chronic disease is surprisingly high. Ninety-one members of the Kings County Medical Society, as an experiment, submitted to health examinations. The ages ranged from thirty-six to fifty years. Eighteen per cent of the ninety-one had minor defects requiring hygienic correction or minor dental or surgical attention. Eighteen per cent required medical supervision as well as hygienic correction and 4.5 per cent had advanced physical impairment requiring systematic medical or surgical care.

Craig reports a health survey of 5,706 police and firemen of Philadelphia. Nearly 2 per cent had some serious cardiac disease, over 2 per cent pulmonary tuberculosis, 5 per cent had albuminuria and nearly 1 per cent showed glycosuria. The majority of these men considered themselves well. Twenty-four per cent were overweight by 20 per cent or more, 2 per cent had bilateral nasal obstruction, 17 per cent had diseased tonsils, and over one-fourth had obvious dental defects. Arterial hypertension with systolic blood pressure 160, or over, was present in nearly 6 per cent of these supposedly normal individuals

Of the 3,764,000 young men examined for the draft, 47 per cent had physical defects of sufficient importance to note on the reports of the examination, and 21 per cent were found physically unfit for all military service.

In 1909, the Metropolitan Life Insurance Co. began an active campaign of health education among its millions of industrial policyholders. This was supplemented with nursing care of those who were acutely ill. The death rate dropped about one-third in the next ten years. There was an increase of eight and one-half years in the life expectancy. Tuberculosis has declined 50 per cent among those insured in the Metropolitan Life Insurance Co. This is better than the general decline.

We must acknowledge that little has been accomplished for the patient after defects have been discovered, unless the patient is given proper advice toward remedying them or toward mitigating their evil results. This requires a detailed

study, not only of the patient's physical condition but of his daily routine, habits, and hygiene. That in practice the individual does profit by having such an examination is indicated by the statistics just quoted. One insurance company which has offered free physical examinations to its policyholders, with advice based upon the examination, calculates that already there has been a return and a profit of 200 per cent on their investment, due to savings in mortality. If a business organization finds this much value in the giving of free medical service to its policyholders, surely a routine physical examination should have a corresponding value to the individual. This value would be further increased when the service was individualized.

The educational value and the tremendous opportunity to the family physician for promulgating health and hygiene are obvious. When a patient who has been thoroughly examined by his physician does develop some acute illness, the physician will undertake the treatment with much greater confidence because he is already acquainted with the terrain over which he must work.

While recognizing the undoubted value of periodic health examinations, we must at the same time realize certain dangers and limitations of the procedure. Perhaps the greatest of these is the risk of mental trauma to the abnormally introspective or neurasthenic, which might make invalids of previously healthy individuals.

A centipede was happy, quite,
Until a frog in fun
Said, "Pray, which leg comes after which?"
This raised her mind to such a pitch
She lay distracted in the ditch,
Considering how to run.

Walsh points out that Pasteur did some of his greatest work, years after his first stroke of apoplexy. Had his physician insisted upon a vegetative existence, after the first illness, the world would have lost much of what Pasteur was able to give.

Each case must be individualized and must be handled with a degree of discretion and judgment which the physician alone has been in a position to acquire.

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—*W. T. V.*

BOOK REVIEWS

(Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.)

*International Clinics, March, 1925**

THIS follows the customary procedure of the series by devoting articles to diversified features of the practice of medicine. The classifications found in the volume are *diagnosis* and *treatment, mental disturbances, surgery, toxicology* and *"progress of medicine for 1924."*

Dr. Barker's article on the treatment of the psychoneuroses is of interest for comparison with similar articles by the author written in years past and in tracing developments and changes in the author's method. Two articles on urologic problems and pyuria give helpful information concerning the simpler methods of urologic study which may be applied by the general practitioner. It is regrettable that these two articles appear to have been borrowed nearly verbatim from works of another author. Seventy odd pages have been contributed by Henry W. Cattell and Jas. F. Coupal in which they review the progress of medical science for the year 1924. This makes a very convenient reference appendix.

Chemistry of the Blood in Clinical Medicine†

THIS is a British contribution and reflects the British attitude toward our present problems of blood chemistry. It is not a laboratory manual but a monograph presenting the knowledge of today in a systematized form. One is impressed that the majority of contributions on this subject, particularly the pioneer work in blood chemical methods, originated in the United States.

The author first presents the chemical constitution of normal blood, followed by a concise, clearly presented study of acidosis and alkalosis. The chapter on blood sugar is presented in the usual manner but that on nephritis reflects more nearly the continental attitude. The author divides the nephritides into azotemic and hydremic. He discusses the protein metabolism and the salt and water metabolism in these two types. He presents only those tests of kidney function which are used by preference in England, namely, the Ambard coefficient and Addis' ratio. The phenolsulphonephthalein test is not presented. Chapters on tetany, rickets and gout are brief but to the point. "Anoxemia" is divided into the arterial type, stagnant type and

*International Clinics. A quarterly of illustrated clinical lectures and especially prepared original articles. By leading members of the medical profession throughout the world. Edited by Dr. Henry W. Cattell. i, 35th series, 1925, J. B. Lippincott Co. Philadelphia. Cloth. Pp. 301.

†Chemistry of the Blood in Clinical Medicine. By O. L. V. de Wesselow. M.B. (Oxon.) F.R.C.P. (London) Cloth. Pp. 255. Price $4.50. William Wood & Co. N. Y., 1925.

anemic type. Oxygen therapy is of course most satisfactory in the arterial type but the author observes that in the anemic anoxemia, oxygen administration may tide the patient over an emergency. Other pathologic conditions discussed are hydremia, anhydremia and glycemia.

A short appendix presents briefly the usually accepted laboratory methods.

The volume is a contribution of worth and should be of value to all those who directly or indirectly encounter the pathologic conditions which the author discusses.

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*An Approach to Social Medicine**

THE author presents a contribution to the science of social welfare which will be found to have great intrinsic worth. His viewpoint is that of the psychiatrist. He points out a number of glaring faults not only in present-day customs and repressive influences but also in many of the recent procedures in dispensing social service. "The amateur social doctors are like the amateur physicians—they always begin with the question of remedies and they go at this without any diagnosis or any knowledge of the anatomy or physiology of society."

The book in its broadest sense becomes an analysis of human character and human behavior, particularly in their relationship to the community. This analysis is primarily biologic so that as its exposition develops, we can clearly follow and understand abnormal mental and emotional reactions in the individual. It is on an understanding of the causes for these reactions that the author bases his suggestion for more intelligent treatment or control.

The sex impulse being one of the greatest driving forces, receives detailed consideration and the psychology of this impulse is followed as it changes from the lower animal through the cave man to the present day extremely complicated social machine. The book necessarily forces a more tolerant attitude upon the reader, but this alone would be not without danger were it not that the author completes his presentation by suggesting in a general way the proper procedure for dealing with the problems. "Of all avenues of approach to the conflict between reproductive and social tendencies the one offering at the present time the broadest perspective appears to be that through which youth is passing to take command of new positions developing on the sites of deteriorated traditions. Candidly this means the evolution of a closer comradeship between men and women, based on sexual desires more equally distributed between the sexes, with a more intensive physical development and a higher spiritual organization, casting into the discard the exploded notion that human sexual contact has for its sole purpose reproduction. While a single standard of morals is to be insisted upon for both men and women it must be recognized that at the present time the

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*An Approach to Social Medicine. By Francis Lee Dunham, M.A., M.D., Lecturer on Social Medicine, Johns Hopkins University. Cloth. Pp. 242. Price $4.00. Williams & Wilkins Co., Baltimore, 1925.

sexes do not share this responsibility equally, the burden falling far more heavily on the continent male.

"Whatever contributes, therefore, to evolve from the 'ladylike' type of Victorian neurasthenic, whose deterioration was due largely to masculine ideas of feminine inferiority, a higher model of comrade, is worthy of widest adoption.

"Instead of segregating the semidependent members of society at state expense, a better policy now advocates their early individual training and continued guidance in the community. This plan shows favorable results when limitations imposed by inferior intellectual capacity are recognized socially and correlated with the individual's conduct capabilities

"Unfortunately community assistance partakes too much of benevolence, sentimentally dispensed, or of coercion inducing pauperism, and it lacks efficiency in dealing with a social group that always may be semidependent and childlike. Recreation centers, the family wage system, social insurance and health protection, are undoubtedly more rational means of treating 'feeblemindedness' in its higher grades than segregation and sterilization."

At the opposite extreme the author asserts that individuals of unusually keen intellect are often stifled in childhood by standards of mediocrity and suggests that in the future these may be given greater opportunity for the exercise of their intellects.

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*General Systematic Bacteriology**

THIS work deals primarily with two phases of bacteriologic systematization, the classification and the nomenclature of microorganisms. The author stresses the difference between the two, remarking that while naturally interrelated they may be altered individually. He presents a brief history of the bacteriologic classifications so far proposed and proceeds to discuss the codes of nomenclature and their relationship to the problems of bacterial terminology. Following this is an alphabetical list of all of the names which have been used by various authors to designate bacterial subgenera, genera or higher groups. The classification suggested by the author is based in part upon the international botanical code.

This work differs from Berger's *Manual of Determinative Bacteriology* in two chief essentials. The classification recommended by Buchanan is not entirely the same as that recommended by Berger and his coworkers, and while Buchanan's book is a monograph on classification, Berger's is in addition a key to the identification of bacteria.

These two volumes represent the latest and most comprehensive efforts to present appropriate classifications of bacteria. Their finality must await the judgment of those bacteriologists who will weigh the practicability of their application.

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*General Systematic Bacteriology, I. By Dr. R. E. Buchanan, Iowa State College, pp. 597, Cloth. Price $6.00. Williams & Wilkins Co, 1925.

*A Laboratory Manual of Physiological Chemistry**

A PRACTICAL working manual in which are incorporated most of the recent so-called practical methods of biochemical analysis with the usual laboratory studies customarily made by undergraduate students in acquainting themselves with the properties of biochemical substances. Among the outstanding chapters which differ to some extent from the older undergraduate manuals are ones on the relation of the ionic theory to properties of living tissues and fluids, on vitamines and deficient proteins, on the acid-base equilibrium in the animal body, short chapters on the systematic testing of urine, classification of tests on blood and urine, and an appendix on the best methods for obtaining and preserving test materials.

While written primarily as a student's manual it should be useful as a reference work for the laboratory worker. The system of blood analysis is particularly comprehensive. The Folin-Wu system is detailed as well as methods for the determination of various other blood constituents as sodium, calcium, potassium, magnesium, phosphorus, chlorides, sulphates, acetone bodies, oxygen capacity and blood gases in general.

The Eastern Association of Tropical Medicine†

TRANSACTIONS of the fifth biennial congress at Singapore 1923. The unabridged transactions are classified into papers dealing with malaria, beriberi, ankylostomiasis, leprosy and miscellaneous. The majority of the malaria papers deal with its epidemiology and prevention. Occasional cases of insanity consequent on malarial infection are reported. These, however, are very infrequent. Several papers deal with the curious condition observed among the Malay peoples known as amok, a form of temporary insanity in which the individual suddenly runs about, killing all who cross his path. This is the derivation of the familiar expression "running amuck." It occurs chiefly among Malays, has been occasionally seen in foreigners who have settled on the Malay peninsula, but apparently does not occur in those Malays who have moved elsewhere. The condition appears dependent upon paranoia and paranoiac forms of insanity, upon acute mania and upon epileptic mental states. Latah, another psychosis observed particularly among Malay races, consists of a most unusual state of suggestibility, an acute hypnotic state.

Wu Lien Teh is inclined to the opinion that the original home of the plague is in the great plateau of Central Asia. It is carried endemically in the wild rodents of this region. He emphasizes the importance of the tarabagan, a small rodent first described by Marco Polo, as a carrier.

The variety of subjects covered and the high character of the contributions makes this a desirable volume for the student of tropical medicine.

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*A Laboratory Manual of Physiological Chemistry. By Elbert W. Rockwood, M.D. Ph.D., and Paul Reed Rockwood, M.D. Illustrated. Pp. 413. Fifth edition, Cloth. Price $4.00 F. A. Davis Co. Philadelphia, 1924.

†The Far Eastern Association of Tropical Medicine. Transaction of the Fifth Biennial Congress held at Singapore, 1923. Edited by Dr. A. L. Hoops and Dr. J. W. Scharff. Illustrated. Pp. 973. Cloth. John Bale Sons & Danielsson, Ltd., London, 1924.

*Handbook of Bacteriology**

THE author has set out to provide a handbook, particularly for students in which the so-called practical aspects are alone incorporated. He has succeeded well. The book is not purely bacteriologic in its treatment but covers phases of immunology and serology. Briefly, it may be described as a volume which covers in condensed form the field which is so comprehensively surveyed by Kolmer in his *Infection, Immunity and Biologic Therapy.* In the portion devoted to systematic bacteriology the alternative nomenclature is that recommended in Berger's *Manual of Determinative Bacteriology.*

The illustrations are unusually well done.

International Conference of Health Problems in Tropical America†

A COMPILATION of contributions, 71 in all, which taken in toto deal with nearly all phases of tropical medicine. The proceedings should be of value to all who are interested in tropical medicine. While epidemiologic and similar considerations find place in most of the articles the major portion is given over to clinical studies.

The Congress which met between July 22 and August 1, 1924, at Kingston, Jamaica, was the guest of the United Fruit Company and the proceedings have been published by this organization. This is a most laudable instance of the wider interests taken by private business enterprise not only in industrial hygiene but in its contiguous ramifications.

The report has been edited by Dr. M. J. Rosenau.

Laboratory Diagnostic Methods‡

THE book aims to "present in a concise manner the technic of the various laboratory tests without any discussion of their value, limitations, or clinical application in the diagnosis and treatment of disease." The first portion is a revision of Pepper and Coleman's *Manual on Pathology.* The present edition represents the clinical, pathologic, bacteriologic and serologic methods employed in the department of pathology and bacteriology of the Graduate School of Medicine in the University of Pennsylvania. It is designed essentially for hospitals and busy practitioners who desire brief yet comprehensive descriptions of the various tests and methods.

Most of the newer contributions have been incorporated. Such are the Rosenthal test for liver function, the Widal and Abrami liver function test, methods for estimating the digestive ferments in duodenal specimens, preparation of colloidal gold reagent, and manufacture of vaccines.

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*Handbook of Bacteriology. For Students and Practitioners of Medicine. By Joseph W. Bigger, M.D. (Dublin) F.R.C.P.I., D.P.H. Cloth. Pp. 413. Price $5.00. William Wood & Co., N. Y., 1925

†International Conference on Health Problems in Tropical America, held at Kingston, Jamaica, B.W.I. Pp. 1010. Cloth. United Fruit Company. Boston, Mass. Illustrated, 1924.

‡Laboratory Diagnostic Methods. By Dr. John A. Kolmer and Dr. Fred Boerner, Cloth. Pp. 338. D. Appleton & Co., N. Y., 1925.

Kindly report any change of address to the Secretary.

The next annual Convention of the American Society of Clinical Pathologists will be held in Dallas, Texas, April 15, 16, 17, 1926.

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CLINICAL AND EXPERIMENTAL

THE RESISTANCE OF FOWL TO STRYCHNINE*

BY W. J. R. HEINEKAMP, M.S., M.D., CHICAGO, ILL.

THE statement is made quite generally in textbooks of pharmacology[1, 2, 3] that fowl tolerate large quantities of strychnine without toxic effects. In keeping with this, strychnine has been widely used to kill destructive rodents, with the belief that fowl or game which ingest the drug would not be injured.

The present investigation is a study of the resistance of fowl to strychnine. In the first part of the work, both chickens and pigeons were used, but later, because of the cost and the fact that similar results were obtained from the two types, pigeons were used exclusively. Strychnine sulphate was the preparation of the alkaloid used. It was given subcutaneously, and also by mouth in solution and in solid form.

Knowing that the presence of food in the alimentary tract might influence the rate of absorption, the strychnine was administered to some birds after feeding, and to some after several hours of starvation. Since strychnine spasms cause glycogen to disappear from the liver and ultimately reduces the blood sugar, the effect of glucose feeding on strychnine spasm also was studied. The details of each procedure are recorded in the protocols.

METHODS AND RESULTS

A. *Normal Pigeons.*—These were given cracked corn and water *ad libitum.* When the drug was given per os in solution, the dose was dissolved in 5 c.c. of distilled water and introduced directly into the crop through a catheter. When solid strychnine was used, it was given in the form of hypodermic tablets, placed directly into the crop by means of forceps. Fluid was withheld

*From the Department of Pharmacology and Therapeutics, College of Medicine University of Illinois.
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from the birds to which solid strychnine was given. When given subcutaneously, the dose of the drug was dissolved in ½ c.c. of distilled water and injected under the skin in the pectoral region.

It was found after a series of preliminary experiments that the minimum lethal dose when given subcutaneously was about 2 mg. per kilo. When given by mouth the M.L.D. was 23.50 mg. per kilo when in a solution, and 30 mg. per kilo when given in solid form.

B. *Starved Pigeons.*—In this series the pigeons were starved for twenty-four to forty-eight hours previous to the experiment—water being allowed—in order to determine the effect of starvation on strychnine action. Administration of strychnine was the same as in the fed birds, subcutaneously and by mouth in solution or in solid form.

It was found that the M. L. D. subcutaneously was 1.5 mg. per kilo, while in solution per os it was 18.50 mg. per kilo, and 20 mg. per kilo when given in solid form. The M. L. D. in starved fowl is slightly less than in fed ones.

C. *With Glucose.*—Glucose was administered in a 20 per cent solution, 10 to 20 c.c. being given every hour for three hours. Then after a period of four hours, during which time a quantity of the carbohydrate might have been converted into glycogen in the liver, strychnine was given subcutaneously and in solid form as described in section A.

It can be seen from Table I that the M. L. D. under both conditions is markedly increased. Subcutaneously the M. L. D. is 6.75 mg. per kilo, and when given in solid form per mouth it was about 70 mg. per kilo. A solution of strychnine per os was not used after glucose, since the crop was filled with fluid and the drug went into solution quickly.

PROTOCOL I

SUBCUTANEOUS INJECTION; FED FOWL; STRYCHNINE SULPHATE

PIGEON	WEIGHT IN GRAMS	DOSE IN MG. PER KILO	REACTION
1.	285	0.50	Recovery
2.	305	1.00	Recovery
3.	293	1.50	Recovery
4.	280	2.00	Death
5.	300	2.50	Death
6.	315	2.00	Death
7.	302	1.50	Recovery
8.	284	2.00	Death

M. L. D. 2.00 mg. per kilo.

PROTOCOL II

SUBCUTANEOUS INJECTION; STARVED FOWL; STRYCHNINE SULPHATE

PIGEON	WEIGHT IN GRAMS	DOSE IN MG. PER KILO	REACTION
1.	306	0.50	Recovery
2.	297	1.00	Recovery
3.	287	1.50	Recovery
4.	309	2.00	Death
5.	288	1.75	Death
6.	296	1.50	Death
7.	285	1.50	Death
8.	293	1.50	Death

M. L. D. 1.50 mg. per kilo.

Protocol III

Subcutaneous Injection; Fed Fowl and After Glucose

PIGEON	WEIGHT IN GRAMS	DOSE IN MG. PER KILO	REACTION
1.	312	0.50	Recovery
2.	294	1.00	Recovery
3.	306	2.00	Recovery
4.	280	4.00	Recovery
5.	272	6.00	Recovery
6.	298	8.00	Death
7.	304	7.00	Death
8.	268	6.50	Recovery
9.	292	6.75	Death
10.	287	6.75	Death
11.	280	6.50	Recovery
12.	293	6.75	Death

Protocol IV

Drug Given by Mouth in Solution; Fed Fowl

PIGEON	WEIGHT IN GRAMS	DOSE IN MG. PER KILO	REACTION
1.	295	0.50	Recovery
2.	311	1.00	Recovery
3.	306	2.00	Recovery
4.	299	4.00	Recovery
5.	300	8.00	Recovery
6.	305	16.00	Recovery
7.	297	25.00	Death
8.	286	20.00	Recovery
9.	290	22.00	Recovery
10.	297	24.00	Death
11.	293	23.00	Recovery
12.	312	23.50	Death
13.	280	23.50	Death

M. L. D. 23.50 mg. per kilo

Protocol V

Drug Given by Mouth; Starved Fowl

PIGEON	WEIGHT IN GRAMS	DOSE IN MG. PER KILO	REACTION
1.	295	10.00	Recovery
2.	306	15.00	Recovery
3.	311	20.00	Death
4.	299	17.00	Recovery
5.	307	18.00	Recovery
6.	302	19.00	Death
7.	305	18.50	Death
8.	283	18.50	Death
9.	288	18.50	Death

M. L. D. 18.50 mg. per kilo.

Protocol VI

Drug Given in Solid Form; Fed Pigeons

PIGEON	WEIGHT IN GRAMS	DOSE IN MG. PER KILO	REACTION
1.	309	10.00	Recovery
2.	312	20.00	Recovery
3.	304	30.00	Death
4.	287	25.00	Recovery
5.	295	28.00	Recovery
6.	283	29.00	Recovery
7.	298	30.00	Recovery
8.	308	30.00	Death
9.	300	30.00	Death

M. L. D. 30.00 mg. per kilo.

PROTOCOL VII

DRUG GIVEN IN SOLID FORM; STARVED FOWL

PIGEON	WEIGHT IN GRAMS	DOSE IN MG. PER KILO	REACTION
1.	304	10.00	Recovery
2.	310	20.00	Death
3.	296	18.00	Recovery
4.	284	19.00	Recovery
5.	278	20.00	Recovery
6.	298	20.00	Death
7.	283	20.00	Death

M. L. D. 20.00 mg. per kilo.

PROTOCOL VIII

DRUG GIVEN IN SOLID FORM TO FOWL FED WITH GLUCOSE

PIGEON	WEIGHT IN GRAMS	DOSE IN MG. PER KILO	REACTION
1.	297	10.00	Recovery
2.	307	20.00	Recovery
3.	304	30.00	Recovery
4.	297	40.00	Recovery
5.	295	50.00	Recovery
6.	278	60.00	Recovery
7.	296	70.00	Recovery
8.	286	75.00	Death
9.	294	70.00	Death
10.	289	72.00	Death
11.	295	70.00	Recovery
12.	289	69.00	Death

M. L. D. about 70.00 mg. per kilo.

Pigeons that recovered were used a second time only after a period of two weeks, that time being allowed for total excretion of strychnine. The appended protocols show the method of determining the M. L. D.

The accompanying table (IX) summarizes the results obtained.

SUMMARY IX

METHOD OF ADMINISTRATION	FORM OF STRYCHNINE	CONDITION OF FOWL	REMARKS	M. L. D.
Subcutaneous	Solution	Fed	—	2.00
Subcutaneous	Solution	Starved	—	1.50
Subcutaneous	Solution	Fed	After glucose	6.75
Mouth	Solution	Fed	—	23.50
Mouth	Solution	Starved	—	18.50
Mouth	Solid	Fed	—	30.00
Mouth	Solid	Starved	—	20.00
Mouth	Solid	Fed	Glucose	70.00

M. L. D. is expressed in milligrams per kilo body weight.

DISCUSSION

Several explanations have been offered for the disintoxication of strychnine in fowl. Londini[4] believed that cholesterol in some way disintoxicated strychnine. Salant[5] found that when strychnine was added to the contents of the large intestine it could not be identified, stating that the substances present destroyed the drug. Hatcher's findings[6] were at variance with Salant's.

Kobert[7] states that with a large dose of strychnine, from 50 to 75 per cent can be recovered unchanged. While Ipsen (Kobert, p. 1157) believed

that strychnine in the liver was not contained in the cell but in the blood, later work[8, 9] has indicated that strychnine is actually destroyed in the liver or held in loose combination there. Although excretion is rather rapid, strychnine has been found in cadavers even after a period of a year.[10]

It is stated[2] that the increased muscular tone and convulsions cause an increased use of glycogen which[3] disappears from the liver in most animals. Repeated doses of strychnine produce quicker and more lasting results in the frog.[11] This last result may be due to two things: first, an increased susceptibility of the spinal cord, or second, inability of the animal to destroy the poison. However, these authors could not detect strychnine in the control animal. Both factors may play a part in cases where sufficient time is not allowed for complete excretion or destruction of the alkaloid.

The inability of the liver to destroy strychnine may be due to either an overwhelming dose, or to a decreased amount of neutralizing substance in the organ, or to a decreased function of the cells due to lack of nutrition. The first fact is controllable. The second is dependent on the third. The elaboration of a neutralizing substance by the liver cell is subject to the nutrition of the cell.

The fact that the M. L. D. for starved fowl is less than for fed ones seems due to a quicker absorption. In the well-fed animal also, larger doses of strychnine might be administered without toxic effects if the liver has optimum nutrition. Since glucose is most easily and quickly assimilated, we introduced this substance into the crop and found that after a period of six hours the M. L. D., both by mouth and subcutaneously, was markedly increased, see Summary (IX).

The results indicate that pigeons and chickens possess an immunity to strychnine only when it is given by mouth. While the M. L.D. given subcutaneously is relatively higher than the M. L. D. for most laboratory animals, it is lower than that for the guinea pig, frog, and adder.[12]

When this drug is given by mouth the M. L D. is markedly higher. Without doubt the amount of food in the crop is a most important factor. The greater the quantity of food in the crop, the slower the rate of absorption of the drug, and hence the liver, which is perhaps the chief site of disintoxication, is not overwhelmed and filters the noxious material from the blood stream.

The condition of the liver is a second factor and probably the more important. When a fowl is starved for forty-eight hours, the crop becomes empty and the store of glycogen in the liver is called upon to furnish food. Strychnine is more active under this condition. Thus, it would seem that the ability of the liver to disintoxicate strychnine depends to some extent on the glycogen content. This consideration would also explain the fact that hashed liver destroys only a small part of the strychnine mixed with it[9], since the glycogen quickly disappears from excised tissue.

CONCLUSIONS

1. Fowl possess a relative immunity to strychnine only when the drug is given by mouth.

2. The M. L. D. depends on the contents of the crop, the rate of absorption being inversely proportional to the amount of food in the crop and directly proportional to its fluidity.

3. Glucose, presumably by increasing the glycogen content of the liver, enables the animal to withstand a larger dose. This is not understood, but it may be due directly to the glycogen or its elaboration of a neutralizing agent.

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[3]Cushny: Pharmacology, Philadelphia, ed. 8, Lea and Febiger, pp. 283, 284.
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THE BLOOD SEDIMENTATION TEST (FAHRAEUS) IN THE DIAGNOSIS AND PROGNOSIS OF DISEASE*

By M. Bochner, M.B. (Tor.), and H. Wassing, M.D., Paterson, N. J.

HISTORY

In 1918, Robin Fahraeus of Stockholm, working in the Physiological Institute of the University of Kiel, published experiments concerning the velocity of sedimentation in the citrated blood of pregnant women in comparison to the sedimentation velocity of citrated blood in nongravid women and in men.

This has led to a great deal of further investigation of this phenomenon, which corresponds to the crumsta phlogistica of Galen and which is so admirably described by John Hunter in his book, "On the Blood, Inflammation, and Gun-shot Wounds." This great English physician, as early as 1797 speaks of the increased tendency of the red cells to sedimentation which he observed in the shed blood of patients suffering from inflammatory conditions, and that the rapidity of the process was in relationship to the severity of the infection. This phenomenon played a most important part up to the end of the eighteenth century and was almost the only blood symptom to which attention was given. It had been so entirely forgotten in the last decades of the nineteenth century, since Virchow's cellular pathology brought about a complete change in our comprehension of disease, that Fahraeus was wholly unconscious of only having found a better method of observing a

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An abstract from this paper was read by Wassing before the Essex County Anatomical and Pathological Society, Newark, New Jersey, November, 1924.
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phenomenon which had been known to Hippocrates; namely, the appearance of the so-called buffy coat or buffy crust of the Greeks, the crusta inflammatoria on the surface of shed blood of patients suffering from inflammatory conditions which did not secrete itself but remained in the blood cake of healthy individuals.

The nature of the shed blood was the prototype for the ancient humoral pathology (*Fahraeus' Review of the History of the Buffy Coat*). Diseases were supposed to have arisen from the cessation of the normal mixture of the four fluids composing the blood: first, the black bile or melancholia, the dark lower part of the blood cake; second, the blood in the limited sense of antiquity (sanguis), the upper portion of the blood cake; third, the yellow bile, gradually freeing itself (the expressed serum); and fourth, the phlegm. The phlegm did not secrete itself spontaneously in healthy blood, but was present in the blood cake. The Greeks knew that by shaking healthy blood the phlegm could be separated and the whole prevented from clotting. In unhealthy blood, however, the phlegm collected in a more or less thick layer on the top of the blood cake, which was, of course, interpreted as being a consequence of this substance having increased.

Fahraeus has given a beautiful description of the entire history of the buffy coat. Until Hunter, there was only the one theory explaining the appearance of the buffy crust; namely, increase of what the Greeks called phlegm. Hunter, who tried the most ingenious experiments on the buffy coat, as for instance, mixing quick sedimentating blood with serum from slow sedimentating blood and vice versa, came to the conclusion that the formation of the buffy coat depends upon the increased quantity of fibrin and on the increased tendency of the red cells to separation; a conception which conforms to all that capillar-electricity, colloid chemistry, and capillary microscopy brought out regarding this phenomenon in the last five years. How is it to be explained that modern science has not taken up the subject of the buffy crust in its annals, other than as an historical curiosity?

The reaction against the humoral pathology through Virchow's cellular pathology was so violent, that one never gave oneself time to prove the observations of our elders respecting the gross appearance of the blood in its true light. It is quite characteristic that at a time when the appearance of the buffy coat in blood of pregnant women had been forgotten, the increase of leucocytes in the blood during pregnancy was discovered, and so, through this complete discarding of humoral pathology, we can more readily understand why Fahraeus, Linzenmeier and others, who undoubtedly had seen hundreds of Wassermann blood specimens in the racks or ice boxes of their laboratories, taken from patients with and without inflammatory conditions, some showing coagulation of the fluid in toto, while others showed separation of the bluish transparent lymph (Hunter) on the surface, had taken no notice of it. When they first observed the difference in sedimentation velocity of the red cells in pregnancy and inflammatory conditions, it appeared new to them. Only *following* their observation, did they discover that the conception of the blood sedimentation, almost in its modern form, had already been familiar to Hunter.

There is no doubt that for exact laboratory purposes and clinical application only the observation of sedimentation in blood, mechanically prevented from coagulation, can be used and it is to the credit of Fahraeus[1] to have introduced this method, and to Linzenmeier,[2] of Leipzig, for having applied this test in the clinical observation of gynecologic and subsequently of inflammatory conditions in general.

TECHNIC

Prior to discussing the clinical value of the test the following is a brief description of the technic as used by us in the laboratory of the Barnert Memorial Hospital. Essentially, it is a modification of the technic as described by Linzenmeier.[3] We use small tubes (Fig. 1), resembling centrifuge tubes, of 6 mm. inner diameter, which are graduated downwards from the 1 c.c. mark into lines of 3 mm. distance for 18 mm. To this tube is added 0.2 c.c. of a 2 per cent solution of sodium citrate. One c.c. of blood from a

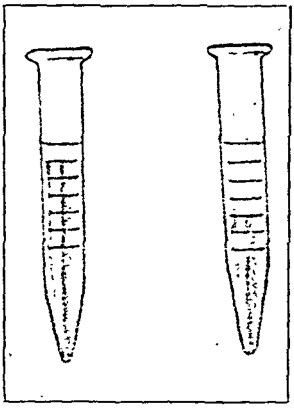

Fig. 1.—Red cell sedimentation after ten minutes; *left,* normal person, sedimentation down to line 3 mm.; *right,* acute appendicitis, sedimentation down to line 12 mm. Tubes in picture are ¾ of natural size.

vein is drawn up into a dry syringe or syringe rinsed with citrate (Fig. 2) and is immediately added to the citrate in the tube, drop by drop, to prevent the formation of air bubbles, until the 1 c.c. mark is reached. The contents of the tube are mixed either by inverting, or better still, by carefully drawing the contents up and down a few times by means of a fine pipette (Fig. 3). This insures thorough mixing without loss of any fluid. For this reason it is superior to mixing the blood and citrate in the syringe. The tubes are now allowed to stand in a vertical position and the reading made when line No. 12 is reached,—in other words, when the red cells have settled down to the line indicating that the height of the column of supernatant plasma is 12 mm. As this test is still in the experimental stage, and no definite standard technic or apparatus adopted, we can readily understand that various investigators use different readings—some very complicated ones. Line No. 18 is the most popular standard at present, but while we do not consider it inferior to line No. 12 there is no doubt that the latter has certain advantages when used as a routine in clinical work. First, the most marked sedimentation velocity

is manifest up to line No. 12; from then on, the sedimentation becomes gradually slower, as observed in the curve, e.g., line No. 12 in fifteen minutes and line No. 15 in one hour. Secondly, in many cases of retarded sedimentation line No. 12 is the lowest index reached within two hours. Thirdly, since the greatest use of this test is in its clinical application, speed without

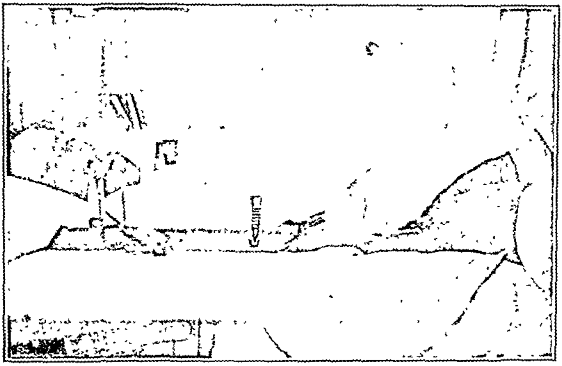

Fig. 2.

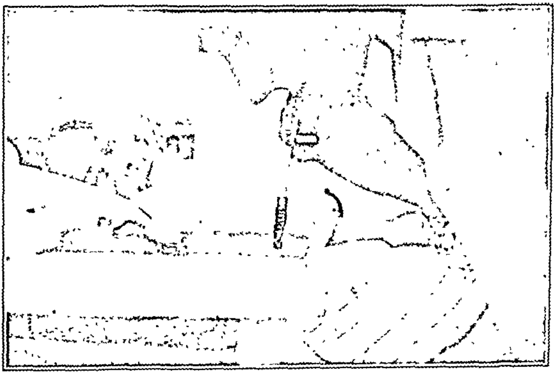

Fig. 3.—Mixing blood with sodium citrate

sacrifice of accuracy and correct interpretation, is manifestly an asset. Normal individuals in line No 12 interpretation are those whose sedimentation velocity is 130 minutes or over; in line No. 18 interpretation those whose sedimentation velocity is 600 minutes or more. In other words, line No. 12 gives as accurate an observation in a much shorter time.

Plotting a curve, with the sedimentation readings as abscissa and the

time of reading as ordinate, gives a very striking picture of the sedimentation velocity at the different stages of the test. To standardize these various methods of sedimentation readings we desire to recommend the use of the sedimentation ratio of the sedimentation velocity *found*, with that of the normal for the same sex, e.g., case of acute appendicitis, female; line No. 12 reached in twelve minutes. Average of healthy women with same method is 130 minutes. Sedimentation Ratio (SR) = 1/13.

The same specimen can be used to repeat the test by remixing and the same readings of sedimentation velocity will be obtained. (An excellent description of the test has been given by Friedlaender,[4] of Detroit.)

Recently, Linzenmeier[5] has developed and advocated the use of a micrometric method wherein a drop or two of blood from a finger is sufficient for the test. Its advantages, where repeated tests are indicated are quite obvious since it eliminates frequent punctures of a vein which at times, are both undesirable and difficult, especially in small children.

THEORY

As is to be expected a number of theories have been propounded to serve as an explanation of this phenomenon. It is doubtful, however, if any one explanation, by itself, can be termed satisfactory. Fahraeus[6] had noticed the rouleaux formation of the clumping red cells and that the sedimentation velocity increased with increase of the aggregation of the red cells. Later, he suggested that this increased aggregation might be due to a new *blood element* which he had observed in freshly examined blood smears. Microscopically, each cell aggregation showed a circular colorless zone in its center, around which the cells clung. This circular zone he has found to be a red cell which has undergone autohemolysis and has acquired this chemotactic-like power to attract other red cells around it and form clumps. He has found the number of these autohemolyzed red cells in direct proportion to the rapidity of the sedimentation. This can be explained by Stokes' law of suspension stability and sedimentation velocity; namely, "the sedimentation velocity of the corpuscle in a suspension of globular elements in fluid is proportionate to the square of their radius." Hence, clumps formed through aggregation of cells will show a quicker sedimentation velocity than isolated cells, and the more numerous these clumps, the more rapid the sedimentation velocity.

Linzenmeier,[7] in his study of the relation of the capillary circulation to blood sedimentation, has observed, that in the capillaries of a healthy individual, a uniform circulation of fine granular bodies is present; whereas, in cases with high fever, or in parturient women, the circulating bodies are coarsely granular and unevenly divided in the blood stream of the capillaries, and there can also be observed a high degree of clumping of the red cells, rouleaux formation and stasis. Can we not, therefore attribute this change in the character of the capillary circulation to these autohemolyzed red cells whose action Fahraeus has observed in freshly examined blood smears?

Höber[8] has shown that erythrocytes wander to the anode and, therefore, carry a negative charge. Schürer and Eimer[9] observed that the corpuscles of healthy individuals wander much more quickly to the anode than in parturient women and inflammatory conditions. The sedimentation velocity has been shown to be associated with a change in the relative proteid compositions of the plasma, i.e., an increase in globulin and a decrease in albumin. Höber[10] states also that different proteids exert different influences on the electrical burden of the corpuscles, i.e., the negative burden is greater in albumin than in globulin solutions. In other words, the negative burden of the corpuscles is diminished and the sedimentation velocity is increased. Linzenmeier has shown that if the plasma is treated with substances which are adsorbents for positive particles, the negative burden of the corpuscles is increased and hence the stability of the blood is raised.

To summarize these findings, we can say that in inflammatory conditions the electrical burden of the cells is diminished, due to an increase of globulin and fibrinogen and resulting in an increased sedimentation. Another factor in the increased S.V., as pointed out by Höber, is that the increased globulin not only diminished the electrical burden of the cells, but, owing to its increased viscosity (the degree of viscosity of proteid solutions being indirectly proportional to their electrical burden), it has also a direct action on the S.V.

It remains to be shown whether the autohemolysis and agglutination of Fahraeus is but an added factor in the process of increased S.V. as is the increased globulin and its increased viscosity. The colloid chemical explanation seems the most plausible. It is interesting to note that Frisch, of Vienna, prior to Fahraeus, had observed the fibrinogen content of the serum to be a very sensitive index of the activity and intensity of an existing tubercular process.

But not only the plasma is responsible for the different S.V. The red cells must also be considered, for the red cells of the horse with its quickly sedimentating blood, hold their speed of sedimentation, partially, when transfered into the plasma of oxen, with their slow sedimentating blood. The difference in the nucleoproteid content of the red cells (34 per cent in horse, 20 per cent in cattle) has been mentioned in explanation of this peculiarity.

CLINICAL VALUE OF THE TEST

The entry of the sedimentation test, like all new procedures in medicine, was heralded with great enthusiasm by many workers,—more so in Europe than in America. And like all new procedures, the enthusiasm it evinced when still in the theoretical and speculative stage, abated somewhat, and became more mature when put through the *acid test* of clinical application. During the past few years, with the intense work done, particularly in Europe, the value of the test from its most useful standpoint; namely, clinical, has been ascertained, and with it came its limitations.

Regarding the sedimentation velocity, we ought never to forget that we are dealing with a phenomenon revealing (as Waugh[11] emphasizes) a *state* or a *condition*, like, for instance, *fever*,—and that this state is just as fre-

quent, or even more frequent, than fever, in pathology. Therefore, it must be understood, that the special value of such a phenomenon is entirely different (it would be unjust to say lower or higher) than the value of such symptoms as eosinophilia, complement fixation, and others, which are characteristic for only one single group of diseases or even for one special disease. The following examples illustrate the point: Given a patient of whom we know nothing other than the presence of an eosinophilia in the blood, will make us suspicious of the presence of one of a group of diseases. Knowing nothing else about the case but that there is no eosinophilia present, this statement will have no value at all, and will seem absurd. If, on the other hand, we know nothing regarding a patient except that he has or has not a fever, the value of these two statements will be about the same, neither one nor the other will promptly help us to make a diagnosis, but both statements will be of general value. From this standpoint it seems false and inconsistent to call fever a symptom, for the translation of the Greek word "symptom" is "accidental finding," which would be the proper term for eosinophilia. For fever, increased sedimentation velocity and similar general phenomena, the expression "state" is undoubtedly a much more corresponding term. (The expression "condition" has been incorporated in the medical terminolgy with a different meaning; namely, "the sum of all outside influences in contradistinction to constitution.")

1. *As an Aid to Diagnosis.*—We find the S.V. increased in all conditions of increased protein catabolism, whenever there are higher products of protein catabolism present or increased in the blood. Consequently, in all febrile conditions; in many inflammatory conditions without fever; in pregnancy after the third month (be it due to increased cholesterol content of the blood or to other physiochemical changes of the serum); after parenteral introduction of a foreign protein; after therapeutic x-ray treatment or influences of radio-active substances upon the organism; in many kidney diseases, due to the presence of certain higher products of protein catabolism in the blood revealing themselves in a rise of a certain fraction of the N.P.N.; in liver diseases where there is a lack of normal protein catabolism present and in tumors where there is resorption of cell products due to disintegration of the tumor. Among other conditions without fever which also show increased S.V. must be mentioned active lues and general paresis and all conditions with decrease of red cells. Runge[12] has noticed an increased S.V. in dementia praecox and only occasionally increased S.V. in melancholia, hysteria, and in psychopathic patients. In arteriosclerosis and in postencephalitis the S.V. was considerably increased.

When considering that in the first years of the Fahraeus test the increased sedimentation velocity has been found in all these mentioned conditions only *successively*, and in the beginning without adequate theoretical foundation, you can imagine the enthusiasm the discovery produced and how much was supposed to be diagnosed from the S.V. The first application of the test to the diagnosis of pregnancy has already failed to keep its promise, for the reaction was only positive with certainty after the third month, and even then, it could be used for differential diagnosis from tumors,

only when there was no disintegration of the questionable tumor, or the presence of an inflammatory condition elsewhere in the body. You see then how restricted here, in reality, is the value of the test.

The attempt to differentiate between appendicitis and adnexitis was, quite obviously, a failure. In the differential diagnosis between inflammatory disease of the adnexa and ectopic pregnancy the sedimentation test is of great value, for in uncomplicated ectopic pregnancy of the first two or three months the sedimentation test will be normal. (If there are slight reactive changes of the peritoneum or intraligamentary hematoma present, it will be slightly increased.) However, all inflammatory diseases of the adnexa show a very rapid S.V. Ruptured ectopic pregnancy will of course be followed by a rapid S.V. In the differentiation of inflammatory and noninflammatory tumors of the pelvis the test will be of value.

Concerning inflammatory conditions in general, we must say the following: "If we have a case with pain, tenderness, and muscular rigidity in the ileocecal region and with 20,000 leucocytes, we will not need the sedimentation test to make the diagnosis appendicitis, and to differentiate from adnexitis it will be of no help to us. It will, however, give us an index of the severity of the condition and possibly of the prognosis. When we see a child with high temperature and pus in the urine, we do not need the sedimentation test to aid us in the diagnosis." Whether the test will ever be of practical use to confirm the diagnosis of general paresis or to make the diagnosis of congenital lues of the newborn (the blood from the umbilical vein shows very slow S.V. normally and in congenital lues a very rapid S.V.), or to differentiate between real or pseudocroup, we do not know. But the test is of very great value in the diagnosis of all those conditions, where there is a suspicion of a latent or an acute inflammatory process and where the leucocyte count is indefinite and the temperature insignificant. In such cases, increase of the S.V. will give us the doubtless decision of the presence of inflammation, as the following case illustrates:

CASE 1.—D. Y., male, forty-two years old, paper hanger, presented himself at the clinic for examination. For the last few weeks while hanging paper he was unable to raise his hand above his head without causing sharp pain in the right lower abdomen. Otherwise he felt well. He gave no history of previous illness other than an attack of abdominal cramps two months previous, which had lasted only a few hours.

Examination revealed a small indefinite and tender mass in the right lower abdomen. Otherwise negative. W. B. C., 9,100; polys, 78 per cent; temperature, 98.4° F.; blood Wassermann negative. S. V., line No. 12 in twenty-four minutes.

As this revealed the presence of a marked inflammatory condition, a second physical examination was made with the purpose of locating possible foci of infection. The findings were negative. A laparotomy revealed multiple adhesions walling off a ruptured appendiceal abscess.

Alexander[13] emphasizes the value of the test in this latter sense (as many authors do) as a diagnostic check in certain cases of chronic arthritis and joint conditions. In early tuberculosis when the temperature, sputum examination, and x-ray do not show anything definite, the sedimentation test is of great value. A *repeated* normal S.V. rules out the presence of active tuberculosis.

sixteen minutes to line No. 12, still indicating the presence of an active condition as also shown by the x-ray.

August 13, 1924, the patient returned from the mountains in greatly improved condition. Temperature was normal, and leucocyte count, 10,000. The S. V. had changed from sixteen minutes to ninety-four minutes.

In acute inflammatory conditions we have found, that where the S.V. is less than ten minutes to line No. 12 (S.R. under 1/13), the prognosis is always poor. This is most marked in pneumonia, empyema, lung abscess, puerperal septicemia, and peritonitis.

In postoperative cases the S.V. shows a gradual return to normal. Should it rise again we are at once warned of an impending complication such as wound infection, peritonitis, or pneumonia. The S.V. in postoperative pneumonia is usually very rapid. Should it be under ten minutes the prognosis is poor.

The following case illustrates this:

CASE 5.—Miss R. G., a saleslady, aged thirty-nine, was admitted July 23, 1924, with diagnosis of chronic cholecystitis. She was operated on next day and an enlarged and thickened gall bladder containing several stones, was removed. On the third day postoperative the temperature rose to 104.3° F.

Examination showed extensive area of consolidation involving lower ⅔ of left axilla and extending to the back. Fetid odor of the breath developed.

Diagnosis of lung abscess confirmed by x-ray August 5, 1924. W. B. C., 18,000; S. V., nine minutes to line No. 12.

Patient died Aug. 6, 1924.

It would seem that the sedimentation test is not only an index of the degree of absorption of catabolic products, but is also an index of the degree of resistance of the host and the virulence of the organism. Else, why should the S.V. be less in empyema, pneumonia, and in caseous tuberculosis, than in septicemia, for instance, where the amount of protein catabolism and absorption in the latter is manifestly much less? Furthermore, in two cases of lobar pneumonia with equal lung involvement, we find one has a S.V. of twenty-two minutes whereas the other has a S.V. of nine minutes. Both have apparently the same areas of absorption, yet the latter dies and we attribute death to a lessened resistance of the host and an increased virulence of the organism. All other factors being equal, the rate of sedimentation seems to correspond *more* to the type of organism and the resistance of the patient, than to the degree of catabolism and protein absorption.

CONCLUSION

In conclusion, we wish to emphasize the value of the sedimentation test in tuberculosis of the lung. Concerning the other possibilities of the test, in medicine and in surgery, the enthusiasm of the first years is subsiding here, as it has in Europe, but in many conditions other than tuberculosis, the sedimentation test has proved its value, and therefore justifies its adoption as an added check to leucocyte count and temperature, subjective and objective findings, and in the diagnosis, course, and prognosis of acute and chronic inflammatory conditions.

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COD-LIVER OIL TREATED WITH MAGNESIUM HYDROXIDE IN THE TREATMENT OF SURGICAL TUBERCULOSIS*

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THE methods which are at the present time available for the treatment of tuberculous abscesses are at the best unsatisfactory or difficult of application in certain cases. Excision is not always possible, incision and drainage results in secondary infection, and aspiration alone is not always curative.

The alternative would seem to be a method which would combine aspiration with the injection of some material possessing an inhibitory or direct antiseptic action on the tubercle bacillus. Such a solution should be very slowly absorbed from the abscess cavity, nonirritating and nontoxic.

During the past two years we have carried out a series of experiments at the Cincinnati Tuberculosis Sanitarium with the object of developing a medium which will have a curative action on closed tuberculous abscesses. Several solutions have been tried, some with no success, others with encouraging results.

In all these experiments it was determined by tests and guinea pig inoculations that the tubercle bacillus was always present and was the sole organism to be demonstrated.

In the course of our work it became apparent that the best medium was a heavy oil which would be slowly absorbed. We decided upon cod-liver oil, basing our choice upon its beneficial effects in general tuberculosis when administered by mouth. Our choice of cod-liver oil was subsequently justified by the recent work of Campbell and Kiefer,[1] who found that it exhibits a definite inhibitory and bactericidal effect on virulent strains of tubercle bacilli.

Cod-liver oil, when injected into tissues unmodified, is too acid to be tolerated, and must be neutralized or alkalinized in order to be safely injected. After trials with various alkalis we determined upon magnesium hydroxide. Equal quantities of magnesium hydroxide and commercial cod-liver oil were boiled together and then centrifuged. We expected a soap to form, but, to our surprise, found that the cod-liver oil was supernatant, unchanged in appearance, and recovered in almost the same amount as when introduced. This oil, however, is alkaline to methyl red and alcoholic phenolphthalein. We have tested it several days after this treatment and found it still alkaline. In this alkaline form the use of cod-liver oil in an abscess cavity is nonirritating and safe.

In our work we have used a commercial milk of magnesia put out by

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one of the well-known drug houses, as it is a suspension of magnesium hydroxide in water. Theoretically, a cold-pressed cod-liver oil would have more antiseptic value, but we have used an ordinary commercial product.

During the last five or six months we have applied this method of treatment with uniform success to the various tuberculous abscesses we have encountered at the sanitarium and elsewhere.

Our success with this solution received an unexpected explanation when the work of Kugelmass and McQuarrie[2] was recently published. These observers have found that cod-liver oil possesses in itself the property of emanating ultraviolet rays. As these are the rays upon which we depend for our results in heliotherapy and quartz lamp treatment of tuberculosis it

Fig. 1.—Film taken by normal cod-liver oil.

would seem that we are placing in an abscess cavity an agent constantly emanating rays of known curative properties. That these rays are of no small intensity is shown by the accompanying film, showing the result of forty-eight hours exposure to cod-liver oil. This film was obtained by inserting an enameled metal dish three-quarters full of cod-liver oil under an x-ray film in its original envelope. The envelope was closed and placed in a lead-lined cabinet for forty-eight hours, when the film was developed. In order to determine whether the treatment of the cod-liver oil with magnesium hydroxide had any effect on the power of emanation, a second similar experiment was performed with the oil so prepared. This film, Fig. 2, shows that here is some slight loss in power of emanation.

It has been stated that the rays responsible for this action upon the photographic plate are ultraviolet, but in none of the published reports have been able to find any competent physical research to substantiate this belief.

It has also been alleged that the action upon the photographic film is entirely chemical, due to vapor emanating from the oil.

Some preliminary experiments have therefore been carried out, with the assistance and under the direction of Gowdy, of the Physics Department, University of Cincinnati. We have established the following facts: that we have here a substance emitting definite rays These rays are probably not of one type, but made up of rays of various wave lengths These rays will not penetrate glass or fusible quartz. They will penetrate paper to a slight degree. Placed in an ionizing chamber the oil shows definite but slight ionizing power. Enclosed in a sealed fusible quartz flask no ionization is obtained.

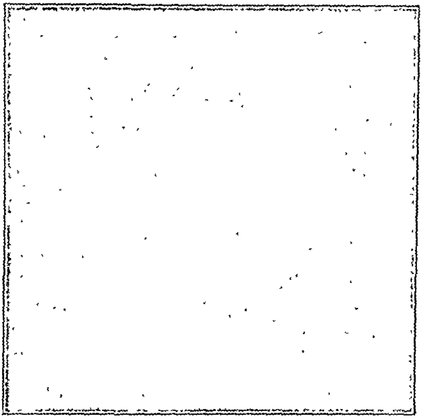

Fig. 2.—Film taken by cod-liver oil after treatment with magnesium hydroxide, showing slight absorption of emanation

The ionizing power of cod-liver oil is not increased by exposure to a mercury quartz lamp.

That the photographic effect is not due to vapor we believe we have determined by directing a current of air upon an x-ray film placed in a light-tight chamber. This current of air has been forced through cod-liver oil by suction. The experiment was continued for forty-eight hours, at the end of which time no effect was noticed upon the developed film.

The density of the photographic picture varies with the mass of the oil and is not a surface effect. For the same surface, denser shadows are produced by greater quantities of oil.

It is interesting to note that Hess in his work on olive oil in rickets has found that when it has been exposed to the mercury quartz lamp the oil acquires a fishy odor, like cod-liver oil.

The physical problem of the exact nature of the rays is not a simple affair and is still under investigation.

The injection of this alkalinized oil into normal tissues has been shown to be harmless and without any irritative or inflammatory sequelae. Rabbits into whose pleural cavities seven and eight cubic centimeters of the fluid have been injected have behaved normally following the injection and when sacrificed ten days after the injection no lesions, macroscopic or microscopic, have been noticed in the pleurae.

We, therefore, believe that the injection of this fluid in a normal pleura is a safe and harmless procedure and by analogy the same would apply to any serous cavity.

Technic.—The skin overlying the abscess and surrounding area is sterilized, and pus aspirated by a long needle or trocar with a caliber sufficiently large to allow of complete emptying of the cavity. It is important to withdraw all of the pus. The abscess is never entered directly, the needle being introduced through at least two inches of surrounding tissue. The amount of pus withdrawn is noted and an equal amount of the oil is introduced through the needle which has not been removed.

To summarize our results in this preliminary report, we believe that we have in alkalinized cod-liver oil a medium which rests upon clinical and theoretical grounds as a safe and efficient treatment for closed surgical tuberculosis. We feel justified in extending its use to empyema, and work along that line is now being carried on.

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A STUDY OF THE STABILITY OF SOME OF THE CONSTITUENTS OF THE BLOOD IN THE TUBERCULOUS*

By Lloyd R. Jones, M.S., Castle Point, New York

FOLLOWING the confirmation of the usefulness of Matefy's reaction for the determination of the activity of a tuberculous process by Kromeke[1] it has been the subject of some experimental work and clinical application in this intsitution for a period of several months.

The technic as originally set forth consisted in the preparation of a fresh 0.5 per thousand solution of aluminum sulphate from a 0.5 per cent stock solution. Of this reagent, 1 c.c. was added to 0.2 c.c. of the serum under test. The tube was well shaken and then set aside for an hour and a half at room temperature after which readings were made; distinct flocculation (not mere turbidity) indicating a positive reaction. In our preliminary studies of this reaction it was at once observed that positive results were being obtained with the blood sera from active cases of tuberculosis and that in individuals giving positive flocculation reactions active tuberculous disease was evident in a high percentage of the number.

Using the technic as outlined above, a series of experiments were made to determine the optimum temperature at which the reaction occurred. Observations were made at three different temperatures; namely, 10° C., 20° C., and 37° C. In working with sera from tuberculous and nontuberculous individuals, it was at once apparent that this reaction is seldom encountered at the lowest temperature even with known reacting sera.

Table I

Tuberculous				*Nontuberculous*			
Patient	10° C.	20° C.	37° C.	Patient	10° C.	20° C.	37° C.
508	−	+	+	R-I	−	−	−
504	−	+	+	R-II	−	−	−
549	+	+	+	386	−	−	−
385	−	+	+	388	−	−	−
408	−	+	+	410	−	−	−
406	±	+	+	10E	−	−	±
550	−	−	+	20E	−	−	− (Luetic)
495	+	+	+	30E	−	−	−
				40E	+	+	+ (Luetic)
				50E	−	−	±
				60E	+	+	+ (Luetic)
				489	−	−	−

At 20° C. and at 37° C., the results are quite parallel, so that it was considered that 20° C. would constitute a satisfactory working temperature for further experiment.

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Considering the amphoteric properties of proteins in solution, by virtue of their amino-acid structure, another project was undertaken to determine the effect of a varying P_H concentration of the flocculating reagent. Diluents with an established P_H concentration of 6.3, 7.0, 7.4, and 8.0 were used in preparing the aluminum sulphate solution. These were then used with known reacting and nonreacting blood sera. As a result it was noted that an acidity or an alkalinity seemed to greatly interfere with the reaction and that for best results the strict neutrality of freshly distilled water (P_H 7.0) was desirable.

In the report of Loeb[2] it is stated that slight concentrations of neutral salts will so affect the potential difference (P.D.) of the electrical double layer surrounding suspended solid aggregates (micelles) that a critical limit may be reached, so that the particles thus altered in their immediate environment will coalesce upon colliding and rapidly settle. In the case of true solutions of proteins, i.e., where the ultimate unit in solution is the protein ion or protein molecule, greater concentrations of neutral salts are necessary to effect flocculation. With this thought in mind another series of experiments was made wherein varying concentrations of the precipitating reagent (aluminum sulphate) were used, as follows: 0.1 per cent, 0.075 per cent, 0.05 per cent, 0.0375 per cent and 0.025 per cent. Upon applying the reaction with these varied concentrations of the reagent to a number of sera from tuberculous and nontuberculous cases it was at once apparent that in dilutions greater than 0.05 per cent the reaction was not obtained irrespective of whether or not the blood serum was from a tuberculous individual. On the other hand, concentrations of the salt greater than 0.05 per cent gave reactions of varied intensity quite regularly with all classes of blood sera.

To summarize the results, it was apparent that the optimum concentration, for clearly defined yet reliable reactions was probably at the 0.05

Table II

Tuberculous patients	Concentration aluminum sulphate %					Nontuberculous patients	Concentration aluminum sulphate %				
	0.1	0.075	0.05	0.0375	0.025		0.1	0.075	0.05	0.0375	0.025
487	+	+	+	−	−	501 (leutic)	+	+	+	−	−
484	+	+	−	−	−	413	+	+	−	−	−
486	+	+	+	−	−	411	+	−	−	−	−
358	+	±	−	−	−	515	+	±	−	−	−
499	+	+	±	−	−	EN	+	±	−	−	−
531	+	±	−	−	−	398	+	±	−	−	−
280	+	+	+	−	−	414	+	+	−	−	−
						412	+	+	−	−	−
						10e	+	+	−	−	−
						20e (leutic)	+	+	−	−	−
						30e	+	+	−	−	−
						40e (leutic)	+	+	+	−	−
						50e	+	+	+	−	−
						60e (leutic)	+	+	+	−	−

per cent concentration point. This observation was clearly in accord with the original report covering the technic of the reaction.

Thinking that the length of the incubation period prior to readings might affect the final result and in that way have a bearing on the usefulness of the test, a group of sera from tuberculous and nontuberculous individuals were subjected to the test and readings made at thirty-minute intervals for a period of two hours.

TABLE III

TUBERCULOUS PATIENTS	INCUBATION PERIOD—HR				NONTUBERCULOUS PATIENTS	INCUBATION PERIOD—HR.			
	½	1	1½	2		½	1	1½	2
385	–	–	+	+	R-1	–	–	–	–
508	–	–	+	+	R-2	–	–	–	–
504	–	±	+	+	10c	–	–	–	+
549	+	+	+	+	20c (leutic)	–	–	–	–
493	–	+	+	+	30c	–	–	–	–
550	–	–	±	+	40c (luetic)	–	–	+	+
219	–	+	+	+	50c	–	–	–	±
220	–	–	+	+	60c (leutic)	–	+	+	+
38	–	–	+	+	541	–	–	–	–
72	–	+	+	+	526	–	–	–	–
63	–	+	+	+	489	–	–	–	–
46	–	+	+	+	161	–	–	–	–
31	–	+	+	+	30	–	–	–	±
54	+	+	+	+	42	–	–	–	–
253	–	+	+	+	72	–	–	–	–
48	–	+	+	+	35	–	–	–	–
182	–	+	+	+					
144	–	+	+	+					
20	–	+	+	+					

While the reaction was observed with most sera from tuberculous individuals in a period of one hours' incubation yet there were a number of known reacting sera that failed to give the reaction at any point prior to an hour and a half of incubation. At two hours incubation distinct flocculation occurred in many of the tubes containing sera from nontuberculous individuals. From these observations it was decided that an incubation period of one and one-half hours at room temperature (20° C.) would constitute the optimum.

In the light of these preliminary findings the test was instituted as a regular procedure upon the admission of patients to the hospital, using 0.2 c.c. of the serum, unheated, the tests all being made on the day following the taking of the specimen. To the serum 1 c.c. of 0.05 per cent solution of aluminum sulphate (C.P. salt dissolved in freshly distilled water) was added, the tube well shaken, set aside at room temperature (20° C.) for an hour and a half at which time readings were made. Distinct flocculation with settling and clearing of the supernatant fluid constituted a positive reaction. Only a few borderline reactions occur; in the great majority of instances the reaction is clear cut and well defined. Heating of the blood serum to 56° C. for fifteen to thirty minutes, the usual procedure of inactivation, interferes greatly with the reaction, almost to the point of complete inhibition.

Up to date, 300 examinations have been made in which clinical findings are available. The results may be classified as follows:

Positive reactions. "Colloid" instability	193
Of the above, patients clinically tuberculous, with B. tuberculosis present in the sputum	174, or 90.1%
Patients with clinical diagnosis of active pulmonary tubercluosis. B. tuberculosis not demonstrable in the sputum	14, or 7.3%
Patients considered to be nontuberculous upon physical examination	5, or 2.6%
Negative reactions. "Colloid" stability	107
Of the above, patients clinically tuberculous with B. tuberculosis present in the sputum	42
Cases with varied diagnoses. B. tuberculosis not demonstrable in the sputum	65

It is quite apparent that many well-defined cases of pulmonary tuberculosis do not exhibit this reaction property, at least so far as one examination is concerned. Limited observations would indicate that the reaction is not necessarily constant, but may vary from time to time. Further investigation in this respect is indicated. The problem has been attacked with the object of determining the incidence of tuberculous disease in individuals giving the reaction of "colloid" instability. Thus far, of the 193 patients giving a positive "colloid" reaction only 5, or 2.6 per cent, have been discharged as being nontuberculous. One of these patients was a diabetic with well-established condition of ketosis and at the time of the test had a fasting blood-sugar content of 440 mg. per 100 c.c. of blood. Under dietary treatment and insulin administration over a period of two months the blood sugar was reduced to 222 mg. per 100 c.c. of blood at which time the "colloid" stability reaction was negative. It is thought that the ketosis may have been responsible for the flocculation in the original reaction.

It is our experience that the "colloid" instability reaction is observed in the blood sera of the tuberculous in many cases and is very probably in accord with the often reported finding that the globulins are increased in amount in the blood stream of the tuberculous.

In our group of nontuberculous individuals it was noted that two of the three syphilitic cases gave a positive reaction. This test cannot be considered as being specific for tuberculosis, but may be of value in the diagnosis of tuberculosis when other diseases can be excluded.

I am grateful for the assistance received from clinicians of this hospital in securing clinical data.

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STUDIES OF THE COLLOIDAL GOLD REACTION USING GOLD PREPARED BY AN ELECTRICAL METHOD*

By Perry J. Manheims, M.D., and Adolph Bernhard B.S., A.M., New York

PREVIOUS descriptions of the colloidal gold reaction have been based either upon the preparation of the gold as originally described by Lange,[1] or modifications of his method.[2, 3, 4, 5, 6, 7] The present paper reports a series of spinal fluid examinations in which colloidal gold prepared by an electrical method was employed.[8]

The method of preparing the gold sol is as follows: The apparatus (Fig. 1) consists of a coil of five hundred feet of number eighteen gauge asbestos covered wire (A), wound on an iron pipe one inch in diameter and one-eighth of an inch thick. Five one hundred watt lamps (B) connected in parallel are used as resistance. One wire leads from the source of the current supply through the lamps into the coil; the other end of the coil is connected to one of the gold electrodes (C). These electrodes are four inches in length, of number eighteen gauge, 100 per cent pure gold wire.† The second electrode is connected to the other pole of the current source.

Preparation of the solution.—Two hundred c.c. of hydrochloric acid solution are made by adding two drops of normal hydrochloric acid to 200 c.c. of distilled water. This gives a solution to about N/10,000. A clean Pyrex beaker may be used to best advantage. The electrodes are immersed in this solution, and steady sparking is allowed to take place for two and one-half minutes.

Stir the solution well and allow to stand overnight; decant, and it is ready for use.

The solution must be stored in Pyrex or nonsol glass-stoppered flasks, and the tubes used in the test should be of the same type of glass.

As is well known, certain precautions should be observed in performing the test in order to exclude errors in technic. Glassware is not to be used for any other purpose, and should be cleaned with aqua regia, then thoroughly washed and rewashed with distilled water. The electrically prepared gold must have a color comparable with a solution of cobalt nitrate-potassium dichromate described by Lee.[9] Its reducing power should be such that the addition of 1.7 c.c. of a freshly prepared 1 per cent sodium chloride solution causes a reduction to a blue color within one hour, and complete reduction (colorless) in twenty-four hours. The gold solution should not be reduced by a normal spinal fluid, while a typical reduction curve should be given by a tabetic spinal fluid. It is essential that the spinal fluid be examined for the presence of blood (using the benzidine method) inasmuch as we have found

*From the Pathological Laboratory of the Lenox Hill Hospital, New York.

†This wire may be obtained from Handy and Harmon, Fulton and Gold St., New York City.

that when blood or blood serum is added to normal spinal fluid a false reduction is obtained. A similar observation was made by Kellert.[10]

The reactions obtained with the use of this gold are recorded as described in "Laboratory Technic, the Methods Employed at St. Luke's Hospital";[9] and for the information of those not familiar with this scale, the following table is given.

COLOR	NUMERICAL COLOR VALUE
Colorless	7
Pale Blue	6
Steel Blue	5
Blue	4
Blue Violet / Violet Blue	3
Violet Blue Red / Violet Red Blue	2
Violet Red / Red Violet	1
Red	0

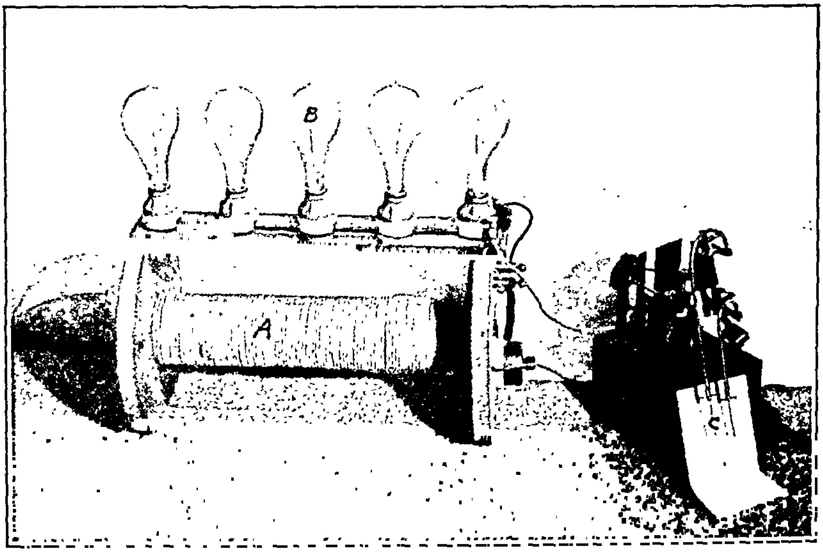

Fig. 1.

Syphilis.—In the twenty-six cases of syphilis (Table I), seven gave a negative curve, a negative Noguchi globulin, and a negative spinal fluid Wassermann. In five of these the blood Wassermann was positive, and in one instance the cell count was 10, no cells being found in the others. Two cases had a negative blood Wassermann. None of these seven cases was diagnosed clinically as syphilis of the central nervous system. Of the nineteen spinal fluids giving curves in the luetic zone, twelve were from cases definitely diagnosed as syphilis of the central nervous system. In nine of these, the spinal fluid Wassermanns were positive, and in seven of these nine, the blood Wassermann coincided. The blood Wassermann was not done in the other two cases. The remaining seven cases of this group giving a luetic curve were all diagnosed as having various forms of syphilis though without clinical signs of cerebrospinal involvement. In these cases the spinal fluid Wassermann was positive

in two instances, while the blood Wassermann was positive in six, not being done in the other case.

When a reduction of the gold in the luetic zone is obtained in cases of syphilis involving other organs than those of the central nervous system, the

Table I

Syphilis

HISTORY NO.	CELL COUNT	GLOBULIN	WASSERMANN		GOLD CURVE	DIAGNOSIS
			SPINAL FLUID	BLOOD		
1394	0	0	0	4+A 4+C	0000000000	Tertiary syphilis
1437	0	0	0	0	0000000000	Syphilis. Chronic nephritis. Autopsy
1387	0	0	0	2+A 3-4+C	0000000000	Subacute endocarditis Right hemiplegia
2083	200	2+	10+A 10+C	4+A 4+C	7777642100	Cerebrospinal syphilis
2466	10	0	–	4+A 4+C	0000000000	Chronic endocarditis Luetic aortitis
2719	90	±	3+A 8+C	2+A 4+C	7773221000	Tabes
2853	20	+	8+A 9+C	4+A 2+C	7777730000	Cerebrospinal syphilis
2095	60	±	0	4+A 2+C	3576631000	Cerebrospinal syphilis
–	140	+	8+A 8+C	–	7774000000	Cerebrospinal syphilis
K	–	–	4+A 8+C	4+A 4+C	2277742410	Cerebrospinal syphilis
5984	220	+	10+A 10+C	4+A 4+C	1127743210	Cerebrospinal syphilis
3223	28	+	10+A 10+C	–	7777765330	Cerebrospinal syphilis
P315	12	+	–	–	7752201230	Cerebrospinal syphilis 5/1/23
P315	30	+	0	–	7774440000	1/2/24
3169	23	0	0	4+A 4+C	7777777522	Secondary syphilis
3244	10	0	0	4+A 4+C	1776520000	Congenital syphilis
3352	0	0	0	4+A 4+C	0000000000	Chronic endocarditis Syphilis of aorta
2213	6	0	0	0	4777744000	Syphilis of central nervous system. Ophthalmic lues
4550	0	0	0	4+A 4+C	0000000000	Tertiary syphilis
4770	0	0	0	4+A 3+C	0357410000	Chronic valvular endocarditis and syphilis
4875	60	3+	8+A 10+C	4+A 4+C	7777743221	Dementia paralytica
5027	8	0	7+A 6+C	4+A 2+C	2236541100	Subacute endocarditis and syphilis
5141	5	0	7+A 5+C	4+A 4+C	2227743111	Dementia paralytica
5216	0	0	0	4+A 4+C	7776542100	Tertiary syphilis
5445	0	0	0	4+A 4+C	0677432111	Facial palsy syphilis
5614	5	0	3+A 3+C	–	7777642110	Tertiary syphilis
2623	0	0	0	0	0000000000	Syphilis—has had treatment

possibility of involvement of the central nervous system must be constantly kept in mind regardless of the cell count, globulin, and the spinal fluid Wassermann. Our experiences in this respect are in accord with those of other authors.[3, 4, 11, 12, 13, 14]

TABLE II

MENINGITIS

HISTORY NO.	CELL COUNT	GLOBULIN	WASSERMANN		GOLD CURVE	DIAGNOSIS
			SPINAL FLUID	BLOOD		
1383	2800	4+	–	–	7777550000	Meningococcus meningitis 5/14/23
1383	5000	4+	–	–	0122773300	5/16/23. After injection of meningococcus serum. Died
1905	270	2+	0	–	1117765220	Tuberculous meningitis. Autopsy
2624	170	+	0	0	11237641000	Tuberculous meningitis. Autopsy
4379	375	2+	–	–	00017774311	Pneumonia—Died Meningitis complication
5100	55	2+	0	0	0017774321	Tuberculous meningitis
3653	400	+	0	0	0000000000	Pneumococcus meningitis Pneumococcus septicemia. Died
6003	2600	4+	–	–	6777777421	Pneumococcus meningitis Pneumococcus septicemia. Autopsy
5392	1300	2+	0	–	0027774321	Tuberculous meningitis 6/21/24
5392	80	4+	–	–	0123776442	6/24/24
3598	130	+	0	–	0000000000	Tuberculous meningitis
5168	155	2+	–	0	7777732100	Tuberculous meningitis 6/3/24
5168	100	2+	–	–	0137743211	6/5/24
5168	160	2+	–	–	0017764311	6/7/24 Died

TABLE III

ENCEPHALITIS

HISTORY NO.	CELL COUNT	GLOBULIN	WASSERMANN		GOLD CURVE	DIAGNOSIS
			SPINAL FLUID	BLOOD		
2938	150	0	0	–	2753200000	Acute anterior poliomyelitis. Autopsy
4744	35	3+	0	2+A 1+2+C	2337776531	Acute suppurative polioencephalitis
5499	0	0	0	0	1137521000	Chronic polioencephalitis
5362	0	0	0	–	0076532100	Chronic superior polioencephalitis
2871	30	0	0	0	2774410000	Acute inferior polioencephalitis 10/11/23
2871	10	+	–	–	346541000	Improved 10/26/23
2871	5	0	0	–	0017761000	11/22/23
5013	9	0	0	0	1127653200	Encephalitis lethargica

TABLE IV

CHOREA

HISTORY NO.	CELL COUNT	GLOBULIN	WASSERMANN		GOLD CURVE	DIAGNOSIS
			SPINAL FLUID	BLOOD		
4116	0	0	0	0	0114555500	Chorea, chronic endocarditis
–	0	0	0	0	0000000000	Chorea
Sch.	0	0	0	0	0000000000	Chorea
4995	0	0	0	0	0000000000	Chorea
1208	10	0	0	0	0000000000	Chorea
4651	0	0	0	0	1266444430	Chorea, chronic endocarditis

A study of Table I shows that there does not seem to be any relationship between the cell count and the gold reaction. This is best demonstrated in a comparison of Case 2083 with a cell count of 200 and a very definite luetic curve, with Case 2853, having a cell count of 20 and exactly the same type and intensity of curve.

Meningitis.—In ten cases of meningitis (Table II), six tuberculous, three pneumococcus, and one meningococcus, all but two gave curves the average

TABLE V

MISCELLANEOUS GROUP

HISTORY NO.	CELL COUNT	GLOBULIN	WASSERMANN SPINAL FLUID	WASSERMANN BLOOD	GOLD CURVE	DIAGNOSIS
1174	2	0	–	2+A 2+C	7774400000	Acute gastritis
1721	10	0	–	–	0000000000	Hemorrhage into cerebrum
3516	12	0	0	0	0000000000	Hemorrhage into cerebrum
3986	28	+	0	0	5777410000	Hemorrhage into cerebrum Edema of lungs
832	10	0	0	0	0000000000	Hemorrhage into cerebrum Hemiplegia
4580	10	2+	0	0	0137775420	Hemorrhage into medulla
2357	5	0	0	0	0012000000	Secondary mastoid operation
1931	0	0	0	–	5577653331	Carcinoma of lung Meningeal metastasis
2138	0	0	0	0	0042100000	Idiopathic epilepsy
2318	10	0	0	0	0000000000	Carcinoma of breast
2389	0	0	0	0	0000000000	Glioma of brain. Autopsy
1724	5	0	0	0	0000000000	Carcinoma hypophysis Metastasis choroidplexus
–	45	+	0	0	7765221000	Migraine
2813	0	0	0	0	0000000000	Chronic synovitis of both knee joints
2902	18	0	0	0	1651510000	Carbon monoxide poisoning
P415	0	0	0	0	0000000000	Influenza
3113	0	0	0	0	3765310000	Acute miliary tuberculosis Autopsy
3372	0	0	0	0	0000000000	Bronchopneumonia
1637	8	0	0	0	7410000000	Hyperthyroidism
3504	0	0	0	0	0000000000	Peritoneal adhesions
3815	0	0	0	0	7650000000	Microcephalus malnutrition
3894	0	0	0	0	7533100000	Rickets
3950	0	0	0	0	0000000000	Chronic nephritis Hemiplegia
4170	5	0	0	0	0000000000	Epilepsy
468	0	0	0	0	0003221000	Cerebro arterial sclerosis
4265	0	0	0	0	0000000000	Cataract
4376	12	+	0	0	0000000000	Left sciatic Neuritis
4568	0	0	0	0	0000000000	Chronic myocarditis Hemiplegia
4627	0	0	0	0	1274322000	Typhoid
4618	0	0	0	0	0027543111	Liver abscess Ovarian abscess
4853	0	0	0	0	0000000000	Traumatic neurosis
892	2	0	0	2+A 4+C	0127431000	Chronic suppur. pleurisy
5048	0	0	0	0	0000000000	Syringomyelia
3808	2	0	0	0	0000000000	Chronic nephritis
Br.	0	0	0	0	0000000000	Migraine
5004	0	0	0	0	0000000000	Serous pleurisy
5173	0	0	0	0	0000000000	Multiple burns
5616	5	2+	0	0	7777765333	Acute gastritis

of which shows the peak (maximum reduction) to be at the center (Tube 5). This type of curve does not exactly coincide with the so-called typical meningitic curve in which the peak occurs at the right of the midline (Tube 6-10). The two cases with negative gold curves had positive globulins and cell counts of 400 and 130 respectively.

Encephalitis.—In the six cases of encephalitis (Table III) all gave a reduction, but no specific curve was obtained. The average for the group showed the peak to be in the luetic zone,[15] but the general type of curve was similar to those obtained in meningitis.

Chorea.—There were six spinal fluids examined from cases of chorea, of which four were negative (no curve) and two gave a reduction. In all these cases the globulin was negative, and in all but one, no cells were found. In Case 1208, there was a cell count of ten and a negative curve.

Miscellaneous Group.—Of the thirty-eight cases studied in this group, twenty-one gave no gold reduction. Two cases of acute gastritis and one case of migraine, with a cell count of 40 and a positive globulin, gave curves in the luetic zone. The other fourteen cases did not give any specific type of curve.[16]

Occasionally a gold curve is obtained when all the other findings, both laboratory and clinical are negative; and under these circumstances when the type of curve is not specific for any given condition, we believe that little importance should be attached to the result.

CONCLUSIONS

1. The colloidal gold reaction in which the gold was prepared by an electrical method was studied in eighty-six cases.

2. The same type and intensity of reactions are obtained with this gold sol as with chemically prepared gold.

3. The presence of blood or blood serum in spinal fluid influences the reduction of the gold sol, and the spinal fluid should always be tested for blood by the benzidine test. If blood is found present, a notation to this effect should be made on the report submitted to the clinician so that he may properly interpret the result.

4. Typical luetic curves were obtained in 85 per cent of the cases of syphilis of the central nervous system.

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AUTOPSY REPORT OF TWO CASES OF THYMIC DEATH DURING SURGICAL OPERATIONS*

BY JESSIE W. FISHER, M.D., MIDDLETOWN, CONN.

MY object in reporting these two necropsies is not so much to recall to your mind the striking pathologic picture or to discuss the disputed etiology but to get an expression of opinion upon some method to detect these cases before they become operating room tragedies. Any diagnostic method, however time consuming or expensive, is worth while if one life can be saved.

That this condition is rare is one of the greatest fallacies. J. E. Benjamin is quoted by Friedlander as finding two hundred and twenty-five new cases in one year, of these 8.4 per cent showed indisputable evidence of enlarged thymus.[1]

It is not within the scope of this report to discuss the various hypotheses brought forward to explain status thymicolymphaticus itself or the factor which causes death following trivial traumas.

Direct pressure of the thymus, hormones, anaphylactic conditions, faulty metabolism, insufficient adrenalin, overfunction of thymus, etc., are some of the explanations given.

Very interesting and enlightening results are coming from the experimental laboratories. The work of Marine, Manley, and Baumann[2] includes observations of the effects of removal of the thyroid, gonads, and spleen as well as of the suprarenals, upon the thymus. These observations have been made on animals covering a period of ten years. The removal of the thyroid, parathyroid, thymus, spleen, suprarenals and gonads, either alone or in combination have been carefully studied. Three hundred and seventy-three animals are reported upon and the results are summarized as follows:

"Thyroidectomy hastens, while gonadectomy delays, but does not permanently prevent involution of the thymus.

"Suprarenalectomy alone not only delays involution of the thymus and

*Read before the Fourth Annual Convention of the American Society of Clinical Pathologists, May 20-23, 1925.
From the Laboratory, Middlesex Hospital, Middletown, Conn

lymphoid tissue but may cause their regeneration. Thyroidectomy prevents this reaction, even, after combined suprarenalectomy and gonadectomy.

"Suprarenalectomy plus gonadectomy is a more powerful stimulus for thymus and lymphoid regeneration than either of these influences alone.

"The combined effect of these two factors results in certain lymphoid and thymus hyperplasia in rabbits, which persists until regeneration of accessory interrenal tissue corrects the physiologic defect. The syndrome thus experimentally produced resembles status lymphaticus and is believed to depend mainly on a partial loss of certain functions of the interrenal and sex glands rather than of the chromaffin tissue.

"The normal and abnormal lymphoid and thymic hyperplasia of infancy and childhood are believed to be manifestations of a functional underdevelopment of the interrenal and sex glands of varying intensity.

"The so-called lymphatic constitution which underlies or accompanies exophthalmic goiter, Addison's disease and acromegaly also appears to be dependent on a partial suppression of certain functions of the interrenal and sex glands."

It is interesting to note that Jaffe's[3] finding of thymus hypertrophy following suprarenalectomy is sustained by this new work of Marine, Manley and Baumann, for they report: "Suprarenalectomy alone, not only delays involution of the thymus and lymphoid tissue but may cause their regeneration," and that "Gonadectomy delays, but does not permanently prevent, involution of the thymus."

No attempt will be made to review the literature which has been thoroughly covered by Marine, Manley, and Baumann[2] to which paper you are referred.

There is no more tragic occurrence from every point of view than the sudden death on the operating table of patients, apparently in the best of health, who are undergoing trivial operations, such as removal of tonsils, etc.

Within three months we have had at our hospital two such deaths both of which showed the classical changes of status thymicolymphaticus postmortem.

The first death occurred in July, 1924, in a Polish boy, J. M., four years of age (one of twins). He was a stocky, pasty looking, rather short necked child without physical stigmata.

Family history was negative except that father had died of pneumonia the previous summer.

Four children of the same family were admitted to the hospital for tonsillectomy. Two older children were operated upon; both took the ether rather poorly, showing cyanosis, but nothing to alarm the etherizer. They also came out of ether poorly but were discharged the following day in good condition.

One subsequently died of what was said to be pneumonia, presumably postoperative, but we could get little information on this subject because of the feeling of the mother in regard to the tragedy. The fourth child was not operated upon on account of the death of the twin.

The preanesthetic examinations revealed nothing to forecast the tragic event. The urine examination was negative.

The child was well under ether (anesthetic, straight ether) and the operation was but just begun, when the patient ceased to breathe. No cyanosis was noted. All the

usual methods including lungmotor, intracardiac hypodermic of adrenalin, etc., were of no avail.

At autopsy the postcervical and elbow lymphatic glands were found palpable. The axillary and inguinal glands were not palpable.

All adipose tissue was a peculiar lemon color.

The thymus gland was firm and lobulated and extended to the fifth interspace. Weight 37.8 grams, left lobe 10 cm. long, right lobe 8 cm. Heart was in systole and the aorta was rather narrow but not specially striking.

No blood, mucus, or other obstruction was found in respiratory tract and lungs were normal.

Tracheobronchial lymph nodes, thoracic duct and lumbar nodes as well as entire lymphatic system showed marked hyperplasia. Glands were firm in consistency and pinkish in color. In other words, they had all the gross appearance of a giant edition of a normal gland.

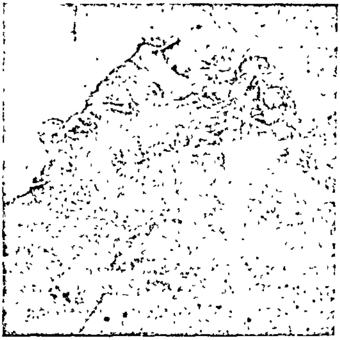

Fig 1.—J. M Thymus gland, weight 37 8 grams.

The mucosae of the small intestine was thickly studded with hyperplastic lymph nodes, varying from pinhead to small shot in size, elevated above the surface like a cauliflower, giving the entire gut a pebbly appearance.

Solitary follicles and Peyer's patches were enormously enlarged. Some of the latter measured 6 to 7 cm. in length.

The large intestine showed similar but not quite such striking changes.

The mesentery was a mass of hyperplastic lymph nodes varying from 1 cm. to 3 cm. in diameter, single and coalescing.

Adrenals showed no gross pathology though they were small and soft.

The spleen weighed 90 grams, and was 10 cm. long by 6.1 cm. wide. On section, the tissue was firm but thickly studded with greyish white masses, the size of a pinhead. (Giant hyperplasia of malpighian bodies.)

Testicles were both descended and normal in size and appearance.

Blood smears taken at autopsy showed small lymphocytes 88 per cent, polymorphonuclears 7 per cent, and large mononuclears 5 per cent.

Wassermann; negative.

Head was not opened.

Microscopic examination showed giant hyperplasia of all lymphoid elements; otherwise, not essentially different from the normal histology.

The second case occurred in October, 1924, three months later.

S. C., aged ten years, Italian, born in America.

The child was rather slightly built, general appearance did not suggest the so-called thymicolymphatic syndrome.

Family history was not of especial significance.

Previous attack of appendicitis a year ago, otherwise personal history without interest.

The boy had been sick for two days before a physician was called. He was ordered to hospital at 10 A.M., but family refused to send him till evening (7:50 P.M.).

On admission he presented a classical picture of ruptured appendix. Tonsils were small, thyroid palpable but no other glandular enlargement noted. Aside from a slight cyanotic hue, there was nothing to suggest thymicolymphaticus.

Blood Count: A.M. 12,200; Polys., 80.5 per cent; S. L., 13.5 per cent; L. L. 5 per cent.

Fig. 2.—J. M. Mesenteric glands.

Fig. 3.—J. M. Peyer's patch, 7 cm. in length.

Blood Count: P.M. 13,400; Polys., 68 per cent; S. L., 28 per cent; L. L., 5 per cent.

Neither urine examination nor Wassermann tests were made.

Anesthesia, began at 9:15 P.M., was straight nitrous oxide; after twenty seconds, oxygen and in two minutes, ether was added.

Pupils were widely dilated.

Pulse, 100 to 120.

As soon as appendix was delivered, ether was discontinued except that on two occasions a small amount of ether was added to nitrous oxide-oxygen mixture when respirations became very slow. A few breaths of ether brought respirations up to normal. Thirty-five minutes after the operation was begun, the patient was pronounced dead.

When closing of peritoneum began, patient's condition was good. Gas was decreased and oxygen increased, so the two were of about equal volume.

When sewing of fascia was begun the pupils began to contract, respiration became slow and shallow and suddenly stopped entirely. At no time was there cyanosis or rigidity.

Artificial respiration, breathing tube, epinephrin intracardially, etc., were used but without results.

The appendix was ruptured and imbedded in a mass of inflammatory exudate with localized hemorrhage and peritonitis.

Autopsy, nine hours postmortem. Aside from the evidence of recent appendectomy, the adenopathy was the most striking feature.

Blood changed to bright red on exposure to air.

The thymus gland covered aorta and base of heart. Weight 29.2 grams; left lobe length 9.5 cm.; right lobe 8.3 cm. The gland was firm, lobulated and pinkish in color.

Mesenteric lymph nodes were prominent. They were irregular in size and shape, varying from size of a shot to 2 cm. in diameter, pinkish in color, firm in consistency, and there was no evidence of necrosis.

Stomach wall was thickened; contents consisted of small amount of bloody mucus. Mucosae showed hemorrhagic areas 2 to 4 cm. in diameter. Entire ileum showed hyperplasia of lymphoid tissue.

Peyer's patches measured from 2 to 3 cm. in length, were nodular and some were hemorrhagic. Hyperplasia was not so marked as in previous case.

Adenopathy was most marked in terminal ileum and from cecum to rectum.

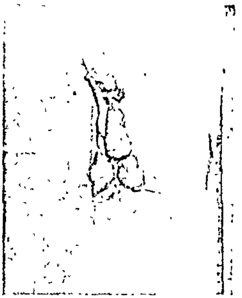

Fig. 4.—S. C Thymus gland, weight 29 2 grams

Spleen weighed 80 grams; trabeculae were fairly prominent; lymphoid hyperplasia was manifested by minute greyish masses throughout the pulp (not so prominent, however, as in previous case).

Suprarenals were small and soft in consistency.

Liver, gall bladder, and urinary bladder were essentially negative.

The kidneys were congested.

Except for rather narrow vessels, there was nothing unusual about the heart.

Larynx, trachea and esophagus were all free from obstruction, blood or mucus, but the bronchi contained a small amount of bloody mucus.

Right lung weighed 200 grams and presented many small areas of atelectasis and three subpleural hemorrhages, irregular in shape; 3 to 4 cm. in diameter, in lower lobe. These hemorrhagic areas were filled with extravasated blood and frothy mucus.

The lower lobe of the left lung was studded with small subpleural hemorrhages similar to those in the right.

Smears and cultures were negative.

Peribronchial glands were similar to mesenteric glands.

Thoracic duct was hyperplastic as were all the subperitoneal and pelvic glands.

Genitalia normal. Testicles normal in appearance.

Brain (weight, 1300 grams) showed tremendous amount of pial congestion with slight edema.

Cerebral vessels were engorged but there were no hemorrhages or other pathology.

The microscopic picture differs in no essential from the normal thymus and lymph glands except in the hyperplasia of lymphoid tissue.

The gonads were not examined microscopically.

The second case was quite unusual as the operative procedures were about finished when death occurred, while in the tonsil case they had just begun.

Both of these cases come under the classification of status thymico-lymphaticus.

Both were males and of foreign parentage.

They were very similar in their adenopathy but differed in that the second patient showed hemorrhagic areas in lungs, stomach walls and Peyer's patches, which probably were due to the effects of infection.

In both cases, the suprarenal glands were small and soft.

Fig. 5.—S. C. Mesenteric glands.

The gonads showed no gross abnormality, but unfortunately were not examined microscopically.

In the second case, the family threatened to bring suit against the hospital, technically, because of autopsy without their permission (coroner had given a verbal order of which their lawyer apparently had no knowledge) but really in the hope of proving malpractice.

The coroner, therefore, held a public inquest at which he courteously permitted the Italian lawyer, engaged by the family, to question the witnesses.

The entire drift of the lawyer's questions were to the effect that the condition ought to have been recognized prior to operation. "If x-rays would show it, why weren't x-rays taken?"

I think the lawyer's implied criticism was justified, though in this particular case, the operation was not one of election.

To those of us who work in small cities, where any unusual case is broadcast by more or less loving (?) friends of the hospital, these cases resolve themselves into three distinct tragedies.

First and foremost, of course, the individual and family tragedy.

Second, the surgical tragedy, i.e., the effect upon the morale of the surgeon, anesthetists and assistants.

Third, the hospital and community tragedy.

Both of these tragedies have been broadcast throughout the county with much adverse criticism of the hospital and the surgeons. The laity just cannot believe that a child can go onto an operating table in good health, and be dead in a few minutes unless some one has grossly blundered.

In large cities, these cases are individual tragedies, not community affairs as they are with us. They, of course, in every instance, except in emergency operations, could be avoided by careful x-rays, and with treatment, these patients might possibly survive the vicissitudes of childhood and become healthy individuals.

REMARKS

A careful physical examination may fail to give the slightest clue to adenopathy.

A lymphocytosis is, of course, suggestive but not pathognomonic especially when cases are complicated by infections as in our second case.

A carefully interpreted preoperative x-ray seems to be the most reliable method of eliminating these casualties though even this fails us when the thymus is not involved.

The Middlesex Hospital has attempted to guard against future accidents by making a rule that all children up to fourteen years of age must have a thymic x-ray before elective operative procedures are undertaken.

If there are any other diagnostic suggestions we will be only too glad to hear them.

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ACTION OF BENZYLAMINE UPON CIRCULATION, SMOOTH MUSCLE, AND RESPIRATION*

By George R. Love, M.D., and J. B. Waddell, M.D., Chicago, Ill.

Barger and Dale[1] mention benzylamine as having a mere trace of sympathomimetic or adrenalin-like action. Since their experiments were quite limited, a more intensive study of benzylamine seemed justified.

Benzylamine was dissolved in distilled water, neutralized with HCl until

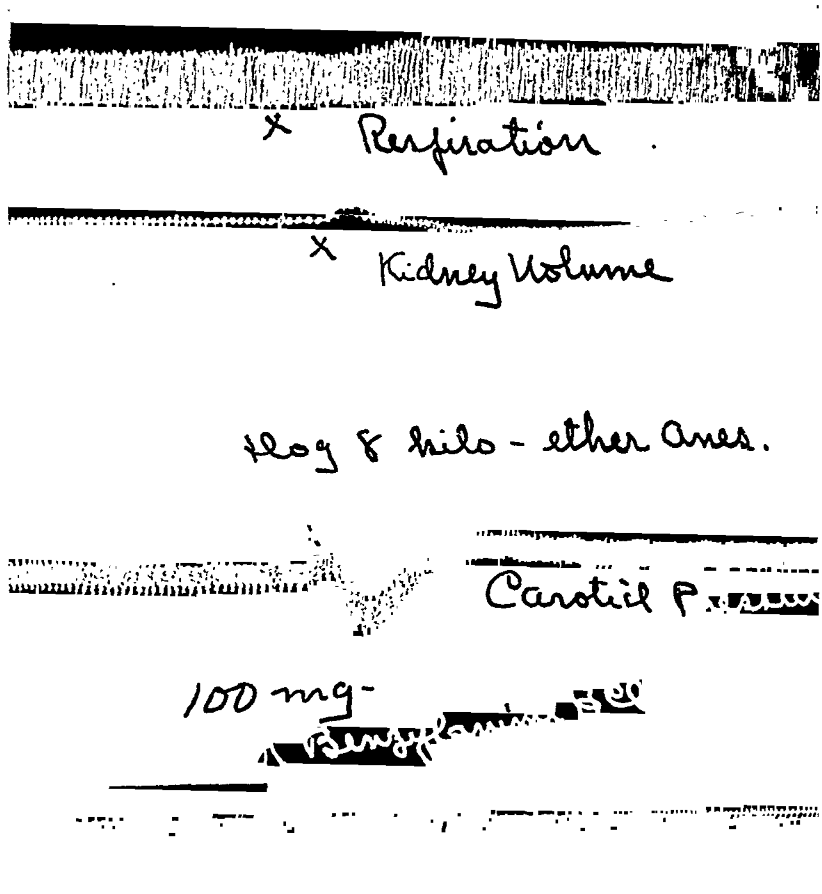

Fig. 1.—A typical effect upon blood pressure, kidney volume, and respiration after the first dose of benzylamine.

barely alkaline (litmus), and diluted with distilled water to make a 5.0 per cent or 10.0 per cent solution in terms of benzylamine.

All in situ experiments were performed upon dogs under light ether anesthesia by tracheotomy.

*From the Department of Pharmacology and Therapeutics of the University of Illinois, College of Medicine.

Received for publication, April 22, 1925.

BLOOD PRESSURE

The intravenous injection of 100 to 200 mg. of benzylamine produces a primary fall of blood pressure which is usually followed by a variable rise above the original level (see Figs. 1, 2, 3). The rise of pressure, when it occurs, lasts only a few moments, at which time the dose may be repeated with practically the same effect. After several injections, the blood pressure assumes a lower level which persists until the end of the experiment, which

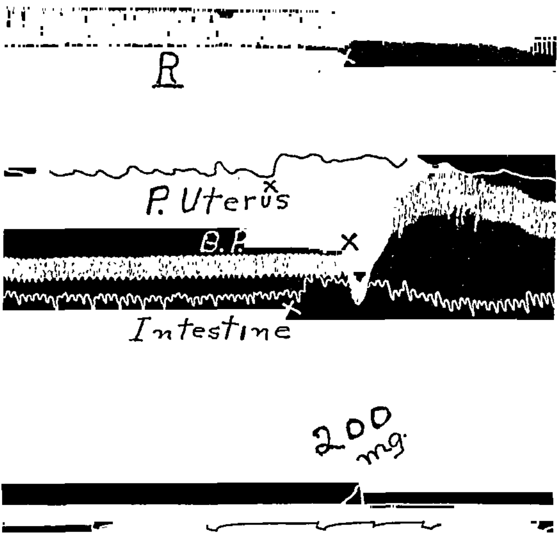

Fig. 2-A.—Dog, 10 kilograms, surgical anesthesia (ether). Note a very slight stimulation of both uterus and intestines. This stimulation is not, however, constant. The rise of blood pressure in this dog after benzylamine is higher than usual.

in this work usually lasted for two or three hours. Larger doses, administered rapidly, cause an abrupt fall of pressure, cardiac dilatation, and cessation.

Occasionally after a large dose, the blood pressure falls, begins to rise, and then suddenly falls to zero. On inspection the sudden fatal fall of pressure appears to be due to acute dilatation of the heart, which is usually followed by ventricular fibrillation (see Fig. 4).

THE HEART

The primary fall of blood pressure is due chiefly to cardiac depression. The proof is as follows:

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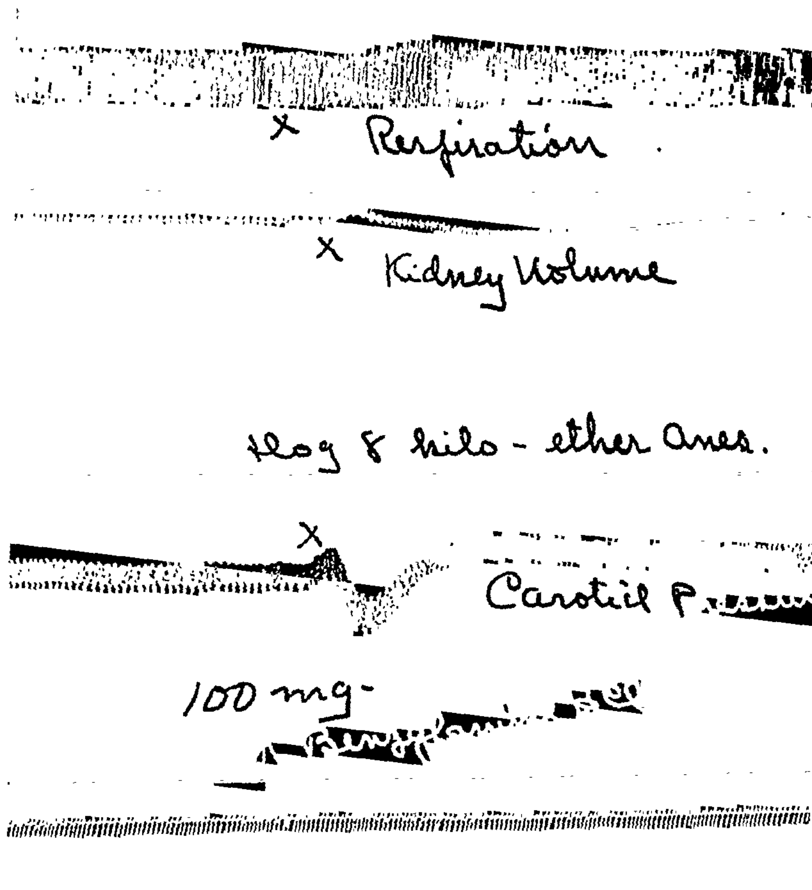

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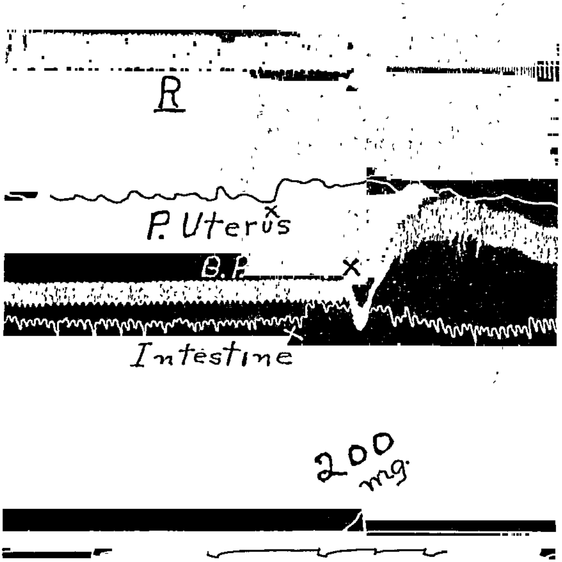

Fig. 2-A.—Dog, 10 kilograms, surgical anesthesia (ether). Note a very slight stimulation of both uterus and intestines. This stimulation is not, however, constant. The rise of blood pressure in this dog after benzylamine is higher than usual.

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THE HEART

The primary fall of blood pressure is due chiefly to cardiac depression. The proof is as follows:

1. The heart dilates (cardiometer) and usually the rate decreases on the fall of pressure.

2. The plethysmograph tracings of abdominal viscera and extremities follow the blood pressure curve downward.

3. The action occurs after section of vagi and administration of atropine.

4. Large doses, as 30 to 50 mg. per kilogram, cause acute dilatation and usually cessation of the heart action.

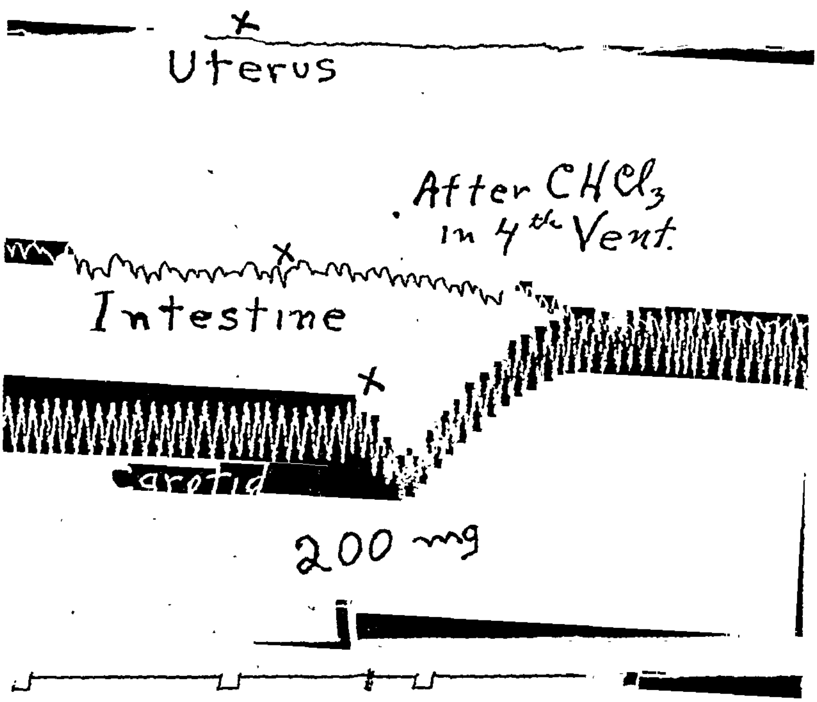

Fig. 2-*B*.—Note that the rise of blood pressure occurs after chloroform injections into fourth ventricle. Uterus and intestines are not influenced by this injection.

BLOOD VESSELS

The plethysmograph tracings of the kidney, spleen, intestines, and extremities follow the blood pressure downward, due chiefly to the cardiac depression. When, however, the blood pressure recovers and rises above the original level, the volume levels sometimes remain below the original level, indicating peripheral constriction. The mechanism of the after-rise of blood pressure is attributed to peripheral constriction and cardiac recovery (see Figs. 1 and 3). This peripheral constriction or rise of pressure occurs after chloroform injections into the fourth ventricle (technic 2) and is therefore not of central origin (see Figs. 2-*A* and 2-*B*).

On perfusion with Locke's solution, 37° C., 120 mm. Hg., the extremities show a definite decrease of outflow after benzylamine. The results from perfusing the kidneys are variable, but more often show a constriction. Several perfusions were made upon extremities before and after ergot and atropine (using epinephrin as an indicator for neuromuscular constrictor paralysis). The benzylamine constriction occurs equally well after ergot and atropine. Excised aortic rings also are stimulated by benzylamine after ergot and atropine (see Fig. 5).

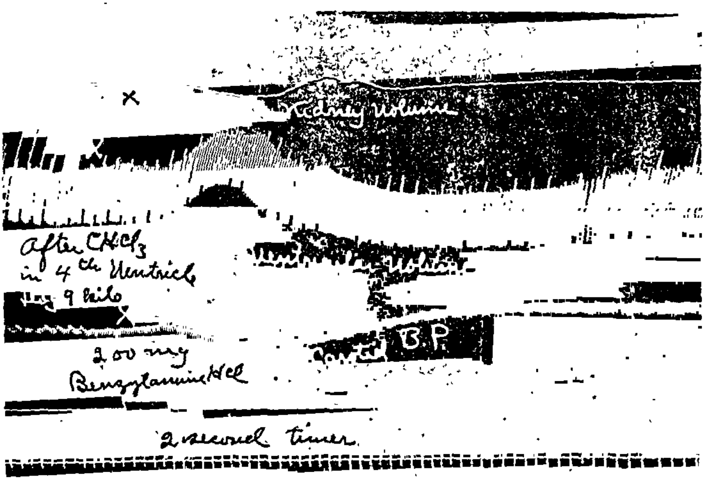

Fig. 3.—Note the acute dilatation of the heart and rapid recovery

PULMONARY CIRCULATION

The pulmonary pressure usually rises from 5 to 30 mm. H_2O following 5 to 10 mg. per kilogram doses of benzylamine, while the carotid pressure falls. This rise may be attributed in part to back pressure, since the left ventricle is more sensitive to cardiac depressants than the right ventricle, and in part to pulmonary constriction. Larger doses produce a simultaneous fall of both carotid and pulmonary pressures, which is of cardiac origin. These effects are illustrated in Figs. 6-*A* and 6-*B*.

On perfusing the lungs under a constant pressure, the introduction of benzylamine causes a definite decrease of the outflow. The excised pulmonary arteries are likewise stimulated by benzylamine. (Technic 4; see Fig. 5.)

The circulatory action of benzylamine may be summarized as cardiac depression and mild constriction of the arteries, both actions occurring directly upon the muscle independent of nerve supply.

STOMACH

The amplitude of the movements and tone of the intact dog stomach is increased by 5 to 20 mg. per kilogram doses of benzylamine (technic 3). The action occurs, though less marked, after atropine (see Fig. 7). After repeated doses of benzylamine the stomach fails to respond.

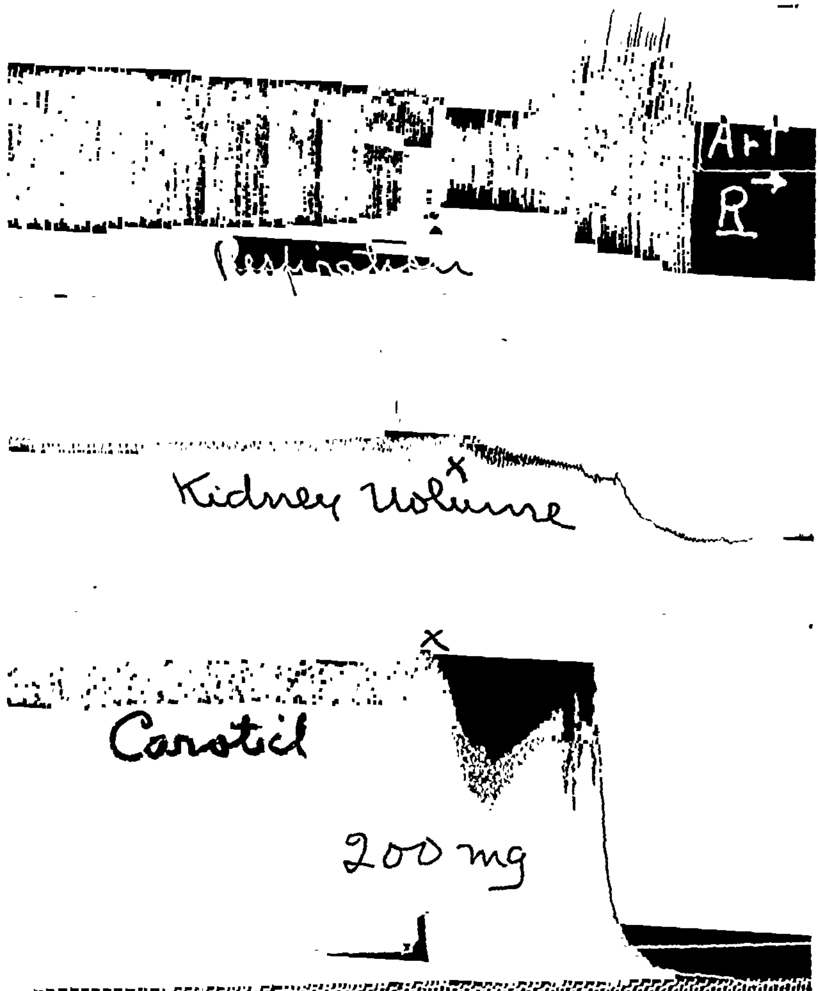

Fig. 4.—Dog, 8 kilograms. The cause of death was ventricular fibrillation. The kidney volume remains low on the recovery of pressure preceding fibrillation.

INTESTINES

Benzylamine has no definite constant action upon the intact dog intestines (see Figs. 2 and 7).

BLADDER

The intact dog bladder is very sensitive to benzylamine. Small doses, 3 to 10 mg. per kilogram, which sometimes do not materially affect the stomach

and circulation, definitely stimulate the dog bladder (technic 3). Larger injections, up to circulatory failure, cause a proportionately greater contraction. This action occurs equally well after atropine.

UTERINE HORNS

The intact uterine horns of dogs, both pregnant and nonpregnant, are occasionally slightly stimulated by benzylamine injections. The action is not, however, definite or constant as upon the bladder or stomach (see Fig. 2).

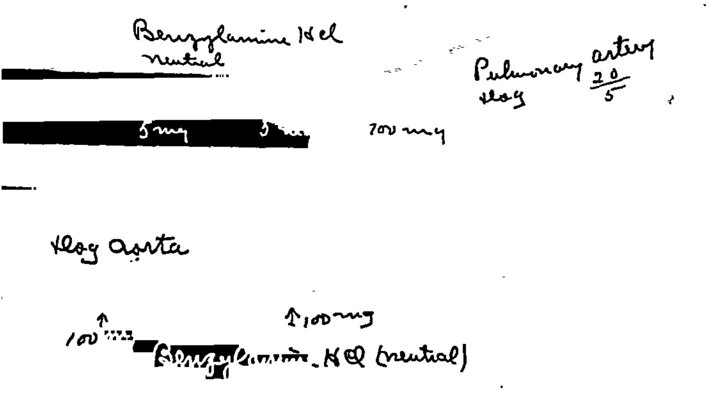

Fig. 5.—The excised pulmonary artery and aorta are stimulated by benzylamine.

EXCISED TISSUES

Excised smooth muscle tissues from the cat, rabbit, and guinea pig were examined by the usual technic, that is, 30 c.c. container, Locke's solution, 37° C., and a constant stream of oxygen or air.

The Cat.—The stomach, intestines (large and small), urethra, uterus (all nonpregnant), and bladder were stimulated by 5 to 25 mg. doses. Of these tissues the bladder is by far more sensitive and vigorous in response to benzylamine. Larger doses, as 100 to 300 mg., usually produce a toxic action in which, although the tissue sometimes does not relax completely, it will not respond to barium chloride. The stimulating action of the smaller doses occurs after administering atropine and ergot.

The Rabbit.—The pregnant and nonpregnant uterus, as well as the other tissues mentioned above, were examined with practically the same results.

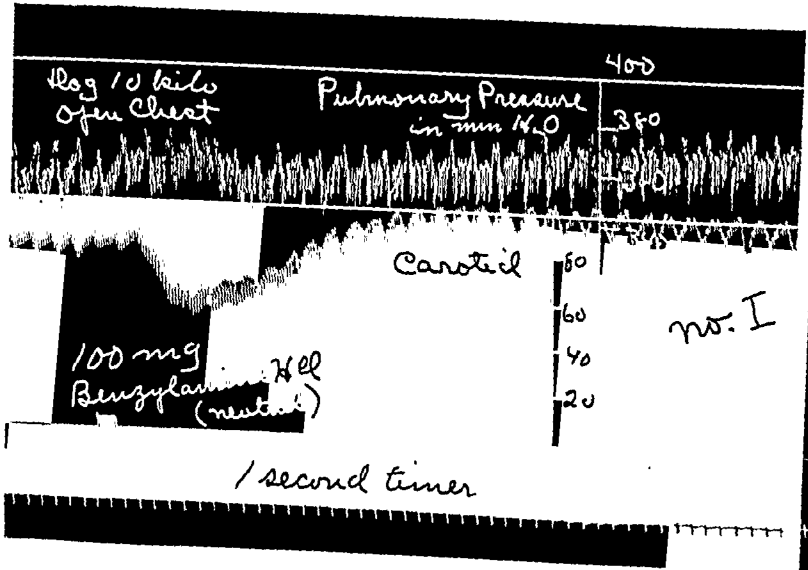

Fig. 6-A.

Fig. 6-A.—Ten mg. per kilogram cause a slight rise of pulmonary pressure and a fall of carotid. Note that pulmonary pressure rises again with the carotid.

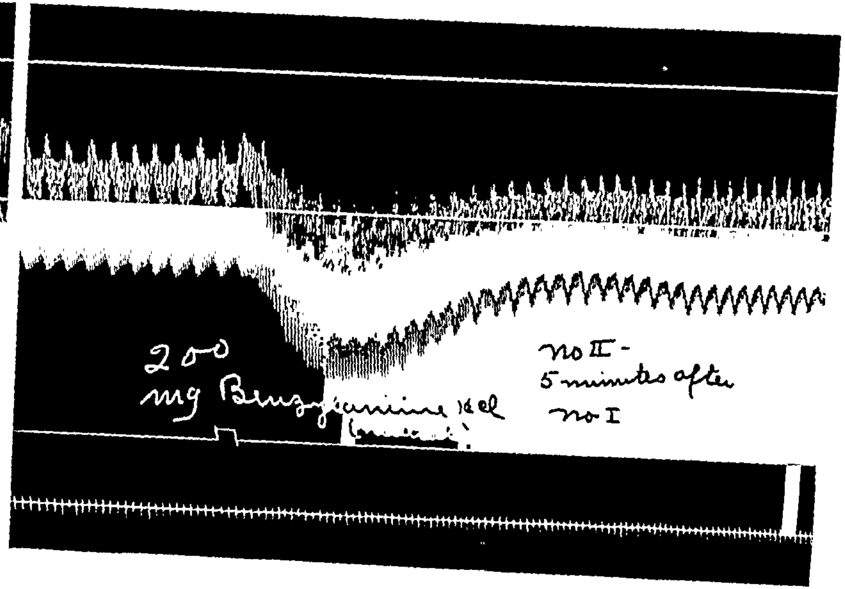

Fig. 6-B.

Fig. 6-B.—A larger dose produces a fall of both pulmonary and carotid pressures, due to profound cardiac depression.

The rabbit stomach and intestines were, however, less resistant to depression from increasing doses than the corresponding cat tissues.

The Guinea Pig.—The stomach, intestines, uterus (pregnant and non-pregnant) are usually depressed by an effective dose of benzylamine. Occasionally these tissues may show a very mild pressor action after a small dose of from 2 to 5 mg. The effective doses of 10 to 50 mg. produce a definite relaxation from which the preparation recovers in a few moments. Larger doses produce permanent depression and loss of barium chloride response.

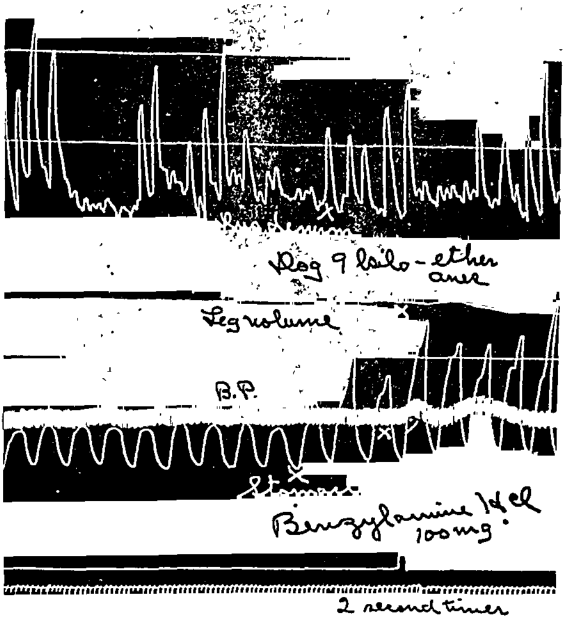

Fig. 7.—The stomach is distinctly stimulated by the first dose of benzylamine. Subsequent injections do not produce such a definite action.

The guinea pig bladder is distinctly stimulated by benzylamine, and is much more resistant to depression from larger doses than the other pig tissues examined. The stimulating action occurs after the administration of atropine and ergot (see Fig. 8).

RESPIRATION

The respiration of dogs under very light ether narcosis is stimulated by the first effective dose, usually 5 to 10 mg. per kilogram. The stimulation is evanescent and may be due in part to the circulatory changes. Repeated

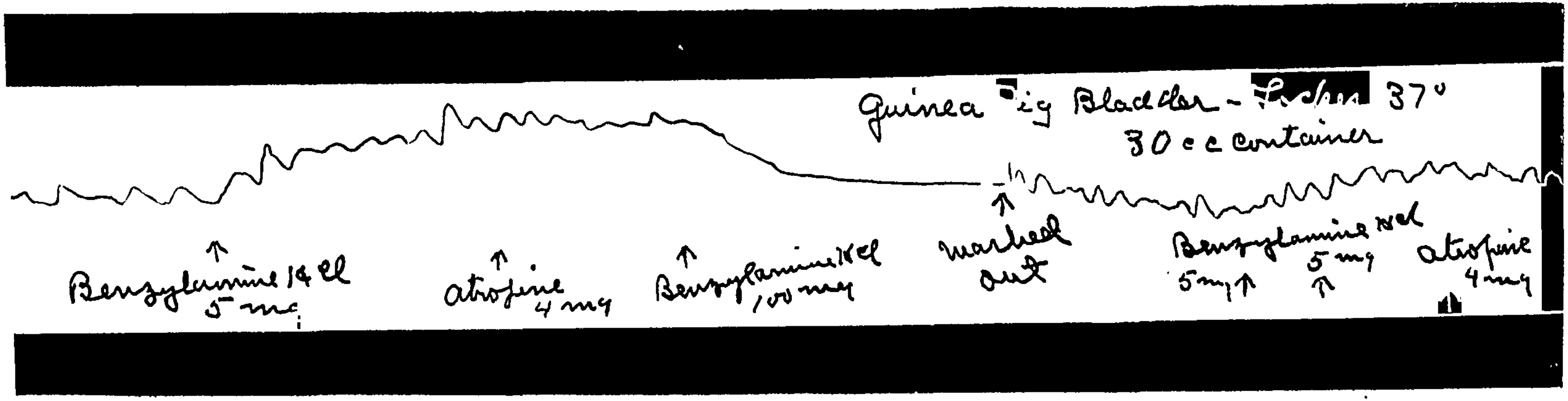

Fig. 8.—Excised guinea pig bladder is distinctly stimulated by benzylamine in small doses after atropine and ergot (ergot not applied on this particular preparation). Large doses cause a depression from which the tissue can recover by washing out.

injections cause a depression and finally a cessation of respiration. Under surgical anesthesia an effective dose usually causes depression. Respiratory failure is the cause of death from increasing doses; however, a large rapid injection of 30 to 50 mg. per kilogram causes a simultaneous failure of both the heart and respiration. The respiration of unanesthetized dogs is not influenced by benzylamine until other actions cause the animals to struggle.

CONCLUSIONS

1. Benzylamine is a cardiac depressant, acting directly upon the heart muscle.

2. The perfused, and excised vessels, including the pulmonary arteries, and usually the intact systemic arteries, are constricted by benzylamine. The action is presumably direct upon the muscle, since it occurs after doses of atropine and ergot.

3. Other excised smooth muscle, such as the stomach, intestines, uterus, and bladder, are stimulated by small doses and depressed by larger doses. The relative action varies in different animals and different tissues of the same animal. The bladder of all animals examined responds more vigorously to benzylamine than other smooth muscle examined.

4. The intact stomach and bladder of dogs are stimulated by benzylamine; they are not depressed by doses which cause circulatory failure. The intact intestine and uterus of the dog are not decidedly influenced by any nonlethal dose.

5. The respiration of dogs under light ether anesthesia is slightly stimulated by small doses and depressed by large doses.

6. Failure of respiration is the cause of death from graduated doses. A large dose injected rapidly may cause death by simultaneous failure of the heart and respiration.

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A CASE OF SQUAMOUS-CELL EPITHELIOMA OF THE STOMACH*

By Porter P. Vinson, M.D., Section on Medicine, and
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ALTHOUGH squamous-cell epithelioma is a common malignant neoplasm in such locations as the skin, lip, tongue, cervix, and bladder, it is rarely found in the stomach in man. It is, however, often found in the stomach in certain of the lower animals, especially the rat. When one considers the frequency with which carcinoma originates in the stomach, it is rather inexplicable that there are not more of the squamous-cell variety among them. Even the gall bladder which, like the stomach, is lined with columnar or

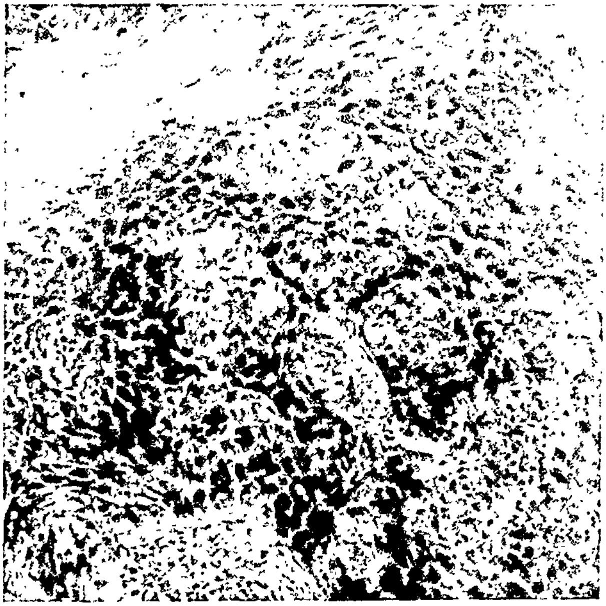

Fig. 1.—Section of stomach showing gland and squamous epithelium, with malignant change in the latter.

secreting epithelium, is the seat of more squamous-cell epitheliomas, although the occurrence of cancer in the gall bladder is comparatively rare.

The question naturally arises of how a squamous-cell epithelioma can originate in an organ lined with columnar or secreting epithelium. The same answer will apply to both stomach and gall bladder; the regenerative cells which normally produce gland or secreting epithelium also produce protective epithelium if necessary, which is shown by the fact that if the uterus becomes inverted and remains so the epithelium of the endometrium changes

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from a gland or secreting type to a squamous or protective type. The following case is an example:

A woman, aged forty-six, was examined in the Clinic, December 30, 1924. She had had an attack of pneumonia at the age of twenty-nine, and following this, obstruction in the lower part of the esophagus to the passage of solid food, unless it was chewed thoroughly. No trouble was experienced in swallowing soft or liquid foods. The dysphagia was thought to be due to a small goiter, but a partial thyroidectomy in 1920 did not afford relief. The trouble continued with about the same degree of severity until January,

Fig. 2—High power magnification of typical area of epithelioma.

1924, when obstruction suddenly became complete. Gastrostomy was performed the following day. The patient had lost thirty-eight pounds and had become quite weak.

Examination revealed considerable emaciation and pallor and a firm nodular epigastric mass. December 31, 1924, a small bronchoscope was introduced into the stomach through the gastrostomy opening and a piece of tissue removed from an ulcerating lesion involving the upper half of the organ. Microscopic examination of a section of the growth showed it to be a squamous-cell epithelioma grade 3 (Figs. 1 and 2).

THE SCHICK TEST AND SCARLET FEVER*

By Thomas G. Hull, Ph.D., Springfield, Ill.

FOR some years past, observations have been made in these laboratories upon the relation of the Schick test to a previous history of scarlet fever. Material for these studies has not been extensive, since accurate histories were not obtainable in most instances.

Park and Zingher[1] have called attention to the fact that while 21 per cent of normal children show positive Schick tests, 45 per cent of children ill with scarlet fever give positive Schick reactions. Zingher,[2] in an attempt to explain the reason for the higher percentage of Schick reactions in children ill with scarlet fever, measles, and poliomyelitis, presents two possible causes.

"1. A destruction of the natural diphtheria antitoxin content during an attack of poliomyelitis or of scarlet fever.

"2. A susceptibility to one of the less contagious diseases indicates that the child is also more apt to be susceptible to other contagious and infectious diseases."

From observations made in these laboratories it would seem that an attack of scarlet fever tended to destroy the diphtheria antitoxin present in the circulation, causing the Schick test to become positive.

Scarlet fever histories have been obtained from 130 persons,—30 adults from the State Department of Public Health, 75 nurses from St. John's Hospital, Springfield, 14 children tested by C. A. Earle, of Des Plaines, and 11 children from Mooseheart, Ill. In Table I, it will be noted that of 107 persons showing no previous history of scarlet fever the Schick reaction was positive in 52 per cent while in 23 persons who had had scarlet fever it was positive in 87 per cent. Three persons who had had scarlet fever gave negative Schick tests. Upon inquiry it was found that several years had elapsed since the date of the disease—twenty years, fifteen years and five years, respectively. There is a possibility that the antitoxic immunity to diphtheria, if destroyed by the case of scarlet fever, had been regained through the intervening years.

The possibility of a greater incidence of scarlet fever among persons showing a positive Schick test was considered. Histories on 24 Schick-tested children were found, who later developed scarlet fever. Of this number 16 were Schick-negative previous to the attack of scarlet fever while 8 were Schick-positive. It was unfortunately impossible to get results of Schick tests after recovery from scarlet fever in this group. It is apparent, however, that the presence of diphtheria antitoxin in the blood has no effect upon the incidence of scarlet fever.

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*From the Division of Laboratories, Illinois Department of Public Health, Springfield, Illinois.

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TABLE I

RELATION OF SCARLET FEVER TO THE SCHICK REACTION

	TOTAL	SCHICK-POSITIVE	SCHICK-NEGATIVE
Previous history of scarlet fever	23	20	3
No history of scarlet fever	107	56	51

While the cases here cited are so few in number that no definite conclusions can be drawn, it would appear that scarlet fever renders the Schick reaction positive. It is hoped that this report will stimulate similar observations by other workers.

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HYDROGEN-ION CONCENTRATION AS A FACTOR IN THE WASSERMANN AND KAHN TESTS*

BY JAMES HARVEY JENNETT, A.B., A.M., KANSAS CITY, MO.

THERE have been many theories and speculations in recent years regarding the part hydrogen-ion concentration plays in bacteriologic and serologic reactions. Since H-ion studies have become so popular, an explanation of many of the heretofore unexplained phenomena is looked for in them. Various workers[1] have suggested that hydrogen-ion concentration may play an important part in complement fixation and some have urged the use of buffered saline solutions in such tests.

When two oppositely charged colloids, as arsenic trisulphide and aluminum hydroxide, are brought together, a precipitate is formed. Can it be that the precipitate in the Kahn test is due to a similar reaction?

When a slight amount of acid is added in the Kahn routine test a false positive is produced.[2] When a slight amount of alkali is added in the Kahn routine test a false negative is produced. Can the difference between positive and negative results in the routine Kahn be due to acidity factors? When a serum globulin solution is brought to the isoelectric point for globulin it will be precipitated. Is the precipitate in the Kahn test the result of bringing the mixture to the isoelectric point of the colloids? These and other similar questions have caused me to wonder if the real difference between a positive and negative serum, in addition to the difference in globulin content,[3] could be a hydrogen-ion variation.

The following experiments were performed to determine the P_H at which these serologic tests are carried out and to find whether there is any differ-

*From the Department of Bacteriology and Preventive Medicine, University of Missouri, Columbia, Mo.
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ence between the positive and negative or whether a variation in H-ion concentration will cause an alteration in the results.

The Wassermann technic was that used routinely in this laboratory, a sheep cell-rabbit amboceptor system. The Kahn test was carried out in detail as described in Kahn's papers.[4] The sera used were from specimens of blood sent in to the Public Health Laboratory for Wassermann tests, and when larger amounts of individual sera were needed, they were taken from patients in town, and from medical students.

In determining the H-ion concentration, both the colorimetric drop ratio method (Gillespie) and the electropotentiometer method were used. Determinations by the colorimetric method were made on specimens dialyzed through collodion sacs against normal saline (0.85 per cent). Determinations by the electropotentiometer method were made on undialyzed specimens and represent the actual P_H of the solutions.

TABLE I

GILLESPIE'S DROP RATIO METHOD FOR DETERMINING THE RELATIVE P_H
(BROMCRESOL PURPLE AND PHENOL RED USED AS INDICATORS)

NO.	MATERIAL	PERIOD OF DIALYSIS	P_H
1	Positive serum 4+	15 minutes	6.7
2	Negative serum –	"	6.9
3	Wassermann mixture 4+	"	6.5
4	Wassermann mixture –	"	6.5
5	Complement	"	6.3
6	Antigen	"	5.7
7	Positive serum 4+	24 hours	8.0
8	Negative serum –	"	8.0
9	Wassermann mixture 4+	"	7.3
10	Wassermann mixture –	"	7.2
11	Complement	"	7.0
12	Antigen	"	6.9
13	Positive serum 4+	"	7.5
14	Negative serum –	"	7.5
15	Wassermann mixture 4+	"	7.1
16	Wassermann mixture –	"	7.1
17	Complement	"	6.8
18	Antigen	"	6.7
19	Antigen not dialysed		5.3
20	Saline (0.85%)		6.1

DISCUSSION

The variations in the accompanying tables are to be expected because of the age of the sera when tested. They were from twelve to seventy-two hours old depending on the time it took for them to be delivered to the laboratory. These are representative sera, however, and the conditions were kept the same as when the daily tests are run. These results are a fair representation of the actual P_H of the mixtures in these routine serologic tests. The results show that there is no difference in P_H between positive and negative sera when examined alone, in Kahn mixtures, and in Wassermann mixtures. The H-ion concentration does not seem to play a very important part in these reactions as long as it is kept in a normal range. Complement fixation and precipitation both take place within considerable range of P_H, the optimum

TABLE II

ELECTRICAL HYDROGEN-ION MACHINE METHOD FOR DETERMINING THE ABSOLUTE P_H.

(The machine used was that in the Physical Chemistry laboratory of the University of Missouri. The electrodes used were of platinum and of calomel. The P_H is calculated from the formula:

$$P_H = \frac{\text{Volts}-0.245}{0.0591}.)$$

NO.	MATERIAL	WASSERMANN RESULTS		VOLTS ON POTENTIOMETER	ACTUAL P_H
		Cholesterol Antigen	Alcohol Antigen		
1	4+ Serum (heated)	4+	2+	0.730	8.20
2	4+ " (unheated)	4+	–	0.713	7.92
3	4+ " "	4+	–	0.719	8.02
4	4+ " (heated)	4+	4+	0.730	8.20
5	– " "	–	–	0.730	8.20
6	4+ " "	4+	4+	0.745	8.46
7	– " (unheated)	–	–	0.745	8.46
8	– " (heated)	–	–	0.742	8.10
9	4+ Wassermann mixture	4+	4+	0.730	8.20
10	– Wassermann mixture	–	–	0.690	7.53
11	Complement (1–10)			0.668	7.15
12	Antigen (1–10)			0.520	4.65
13	Saline (0.85%)			0.537	4.94
14	Completed Wassermann + R B. C. + amboceptor	4+		0.719	8.04
15	Completed Wassermann without R. B. C.	4+		0.727	8.15
16	Completed Wassermann + R B. C. + amboceptor	–		0.701	7.72
17	Completed Wassermann without R. B. C.	–		0.735	8.28
18	Complement 48 hours old			0.681	7.37
1	Fresh positive serum	2+	–	0.714	7.93
2	" " "	4+	4+	0.725	8.12
3	" " "	4+	4+	0.723	8.08
4	" " "	4+	1+	0 737	8.32
5	" " "	4+	1+	0.715	7.95
6	Saline (0.85%)			0.489	4.09
7	4+ Kahn mixtures	4+	4+	0.724	8.12
8	4+ Kahn mixture	4+	4+	0.718	8.00
9	– Kahn mixtures	–	–	0.735	8.29
10	– Kahn mixture	–	–	0.719	8.04

being at about P_H 8. Nearly all serum reactions done in laboratories are done at a P_H of around 8.

Complement fixation occurs from P_H 5 to P_H 9[5] and between these points the hydrogen-ion concentration does not explain or influence the results.

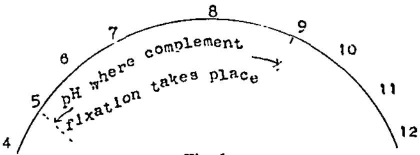

FIG. 1.

The degree of complement fixation rises with the P_H to a certain optimum and then falls again as the P_H becomes too high, the apex of the curve being at P_H 8 (Fig. 1). When false positives are produced in a negative serum by the addition of acid, the buffer effect of the small amount of serum used was over-

come and the solution taken out of the range for complement fixation or precipitate formation. The reaction is now due to excess acid. Blood serum is too highly buffered to undergo any significant change or variation in these tests as far as the hydrogen-ion concentration is concerned.

CONCLUSION

Positive and negative sera, alone and in all the Wassermann and Kahn combinations, show no significant difference one from the other in hydrogen-ion concentration. As long as the P_H is within a certain range it is not a factor in the Wassermann and Kahn tests.

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[1]Mason and Sanford: Jour. Lab. and Clin. Med., February, 1924, ix, 313.
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[4]Kahn, R. L.: Arch. Dermat. and Syph., May, 1922, v, 570.
[5]Morgan: Jour. Immunol., November, 1923, viii, 449.

THE USE OF LIVING VEGETABLE TISSUE FOR SECURING A FAVORABLE GASEOUS ENVIRONMENT FOR ANAEROBIC BACTERIA*

By W. R. Carroll, M.S., Urbana, Ill., and E. G. Hastings, M.S., Madison, Wis.

THE constant stream of papers, presenting methods for the isolation and continued cultivation of anaerobic bacteria, indicates the absence of a satisfactory method. We hope pardon may be granted by the readers hereof if the points suggested do nothing to simplify the present technic.

Two conditions with reference to the gaseous environment of anaerobes seem essential for their best development. First: that no free oxygen shall be present, a condition difficult to obtain with methods of replacement or evacuation. Second: the presence of a small percentage of CO_2. The method of Rockwell[1] who has proved the value of this gas in favoring the growth of anaerobic bacteria, is the only one in which such a condition is obtained.

It occurred to us that living plant tissue in the container, in which plates or tubes seeded with anaerobic organisms are to be incubated, should provide an absence of free oxygen and an amount of CO_2, equal to the oxygen absorbed, since the respiratory coefficient is approximately one. The use of such an agent would thus imply no change in volume of the gas in the containers other than that caused by temperature variations. This would be so slight as not to demand complicated means of closing any vessel contain-

*From the Laboratories of the Department of Agricultural Bacteriology, University of Wisconsin.

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[1]Rockwell, George C.: Jour. Infect. Dis., 1924, xxxv, 581-586.

ing the cultures. Trials with the method have yielded most satisfactory results. Plate culture No. 1 in Fig. 1 was seeded with *B. granulobacter pectinovorum,* an organism very sensitive to free oxygen, and incubated under aerobic conditions. Plate culture No. 2 in Fig. 1 is a similar plate incubated in an atmosphere from which the oxygen had been removed by potato and to which CO_2 had been returned by the same agent. Plate No. 1 in Fig. 2 is a giant colony of *B. granulobacter* which developed in an atmosphere free of both oxygen and CO_2. The usual alkali and pyrogallic acid mixture was used. Plate No. 2 in Fig. 2 was incubated in an atmosphere free of oxygen, but containing CO_2. Potato was the active agent.

The following organisms have given good surface growth under these conditions; various strains of *B. granulobacter pectinovorum, Clostridium botulinum, Clostridium chauvei* and *Clostridium oedematismaligni.*

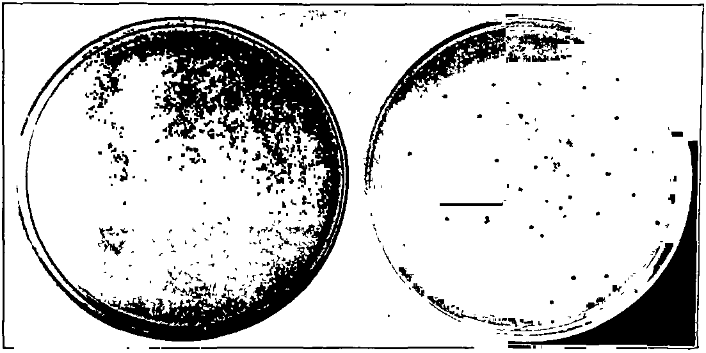


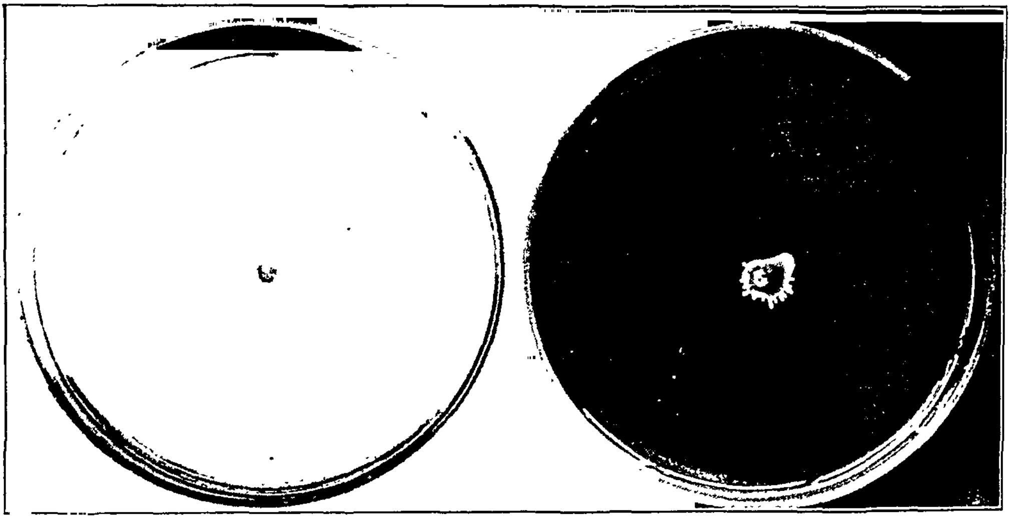
Fig. 1.—Showing dilution cultures of B. granulobacter pectinovorum. *1,* Petri dish No. 1, incubated under aerobic conditions. *2,* Petri dish No. 2, incubated under bell jar with chopped potato.

Tube cultures may be placed in a large test tube in the bottom of which has been placed cubes cut from a well washed and peeled raw potato. A large amount of fresh surface is thus exposed and oxygen is rapidly absorbed. On more completely macerated tissue bacteria present are likely to grow before the end of the incubation period. The large tube may be sealed with a rubber stopper. Since there is no especial change in volume of gas, other than that produced by the cultures or through the growth of bacteria on the potato, the stopper will neither be drawn in nor blown out. An ordinary desiccator or a museum jar with a flat glass cover sealed with plasticine may be used. Tubes or plate cultures may be placed under a bell jar set in a basin or other vessel and a seal of mercury or heavy oil used. In this case the potato is placed in an inner container.

We have little information concerning the amount of potato needed per liter of enclosed air. It is probable that a small amount of tissue will use

up all the free oxygen if time is not to be considered. We have used about fifty grams of potato per liter of air. Germinating oats and lettuce have been used, however, potato seems to be the cheapest and most available material.

Sterile milk to which sufficient sterile methylene blue solution has been added to give a distinct color is an excellent indicator for anaerobiosis. When free oxygen is absent, the milk will reduce the methylene blue.



1. 2.

Fig. 2.—Showing giant colonies of B. granulobacter pectinovorum. *1*, Petri dish No. 1, under bell jar with alkaline pyrogallic acid. *2*, Petri dish No. 2, under bell jar with chopped potato.

The method suggested has, we believe, the following advantages: It is simple, permitting the culture of anaerobes with the apparatus ordinarily on hand in any laboratory; it is inexpensive, the absorbing medium being cheap and available at all times; and it is effective, since it gives the most favorable gaseous environment for anaerobic cultures.

ANTIGENIC VALUE OF VARIOUS TISSUE EXTRACTS IN KAHN TEST*

By Pearl L. Kendrick, B.S., Lansing, Mich., and H. G. Dunham, B.S., Detroit, Mich.

An attempt was made to determine the relative potency of various tissue extracts when used as antigen in the Kahn precipitation test. The problem was of interest from the standpoint of the relative distribution of antigenic substance in various organs. It was also of practical importance since such a comparative study would indicate whether heart muscle, so widely used in antigen preparation, contains more antigenic substance than other tissues—particularly those rich in cellular material, such as liver.

By preparing extracts from various powdered tissues according to a uniform method, such as is used for the preparation of Kahn antigen, and testing these antigens with serums in the Kahn test, it seemed that a relatively true comparison of the amounts of antigenic substance in the different tissues could be obtained.

EXPERIMENTAL

Seven beef tissues; namely, heart, liver, kidney, tonsil, lung, spleen, and brain were employed in the experiments. They were prepared as follows:

Preparation of Powdered Tissue.—The different tissues were removed from the beef as soon as possible after the animal was killed. The tissues were immediately put into ice water and taken to the laboratory for preparation. Each tissue was carefully trimmed with a knife to remove as much fat as possible. Foreign tissue and blood vessels were also carefully removed. The trimmed tissue was sliced in thin layers and washed thoroughly with cold tap water to remove the blood as completely as possible. The washed tissue was drained, minced to a paste, spread in thin layers and dried *in vacuo*. The dried material was then powdered by grinding in a ball mill.

Preparation of Antigen.—An extract was prepared from each powdered tissue according to the standard method for preparing Kahn antigen.[1] (The filtration time during the periods of extraction with ether could not be kept absolutely the same in all cases as some of the tissue (ether mixtures) filtered more slowly than others. The brain tissue gave the greatest difficulty in filtration and in this case it was necessary to resort to a Buchner filter.) Each antigen was titrated according to the Kahn method to determine the amount of salt solution required in its dilution for the tests.

Tests with Serum.—The seven antigens were diluted according to titration and tested for their relative sensitiveness in the Kahn test with 120 serums of varying potency. The results are shown in Table I. These findings indicate

*From the Bureau of Laboratories, Michigan Department of Health, Lansing, Michigan, and from the Bacteriological Laboratories, Digestive Ferments Co., Detroit, Mich.

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that the antigenic extract of heart tissue is the most sensitive of the antigens tested. Next in sensitiveness is extract of liver. Extracts of kidney, tonsil, lung, spleen and brain follow in order,—brain tissue producing the least sensitive extract of all.

Table I

Comparative Sensitiveness of Antigen Extracts of Various Tissues

NUMBER OF SERUMS	EXTRACTS PREPARED FROM BEEF						
	HEART	LIVER	KIDNEY	TONSIL	LUNG	SPLEEN	BRAIN
	REACTIONS SHOWING AGREEMENT						
7	++++	++++	++++	++++	++++	++++	++++
55	−	−	−	−	−	−	−
	REACTIONS SHOWING VARIATION						
3	++++	++++	++++	++++	++++	++++	+
5	++++	++++	++++	++++	++++	++	++
2	++++	++++	++++	++++	++++	++	−
4	++++	++++	++++	++++	+++	+	+
1	++++	++++	++++	++++	++	−	−
2	++++	++++	++++	++++	++++	−	+
1	++++	++++	++++	++++	++	+++	+
1	++++	++++	++++	+++	+++	++	++
3	++++	++++	+++	+++	+	+	−
1	++++	++++	++++	+	−	−	−
1	++++	++++	++	++	+	−	−
1	++++	++++	++	−	+	−	++
1	++++	++	++++	−	−	−	−
3	++++	+++	−	−	−	−	−
1	++++	++	−	+	−	−	−
1	++++	−	++	−	−	−	−
2	++++	+	+	+	+	+	+
1	++++	+	+	−	−	−	−
1	+++	++	++	++	++	++	++
1	+++	++	+	−	+	−	−
1	++++	−	−	+	−	−	−
1	++++	−	−	−	−	−	−
20	++	−	−	−	−	−	−

CONCLUSION

The antigenic substance responsible for precipitation with syphilitic serum is present in various tissues of the body. The quantity available, however, varies with the tissue. Of seven beef tissues tested; namely, heart, liver, kidney, tonsil, lung, spleen and brain, the largest amount of antigenic substance was found in the extract of heart muscle.

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[1]Kahn, R. L.: Serum Diagnosis of Syphilis by Precipitation, Governing Principles, Procedure and Clinical Application of the Kahn Precipitation Test, Baltimore, 1925, Williams & Wilkins Co.

LABORATORY METHODS

THE BACTERICIDAL ACTION OF WHOLE BLOOD AS DETERMINED BY THE HEIST-LACY METHOD*

By B. S. Parks, M.D., M. Sc., Parkersburg, West Virginia

THE announcement by George D. Heist, Solomon Solis-Cohen and Myer Solis-Cohen, of their method to determine the bactericidal power of whole coagulated blood, has apparently opened a new avenue of approach to the study of certain phases of immunity.

The application of this method has given birth to new and improved methods in the treatment of the diseases due to bacteria and their toxic products.

These investigators described a test by which it is possible to distinguish in vitro between animals that are either susceptible or immune to infection with the pneumococcus. They found the same test applicable in distinguishing between animals that are either immune or susceptible to infection with the globoid bodies, as well as infection with meningococcus and other bacteria.

Heist, Cohen, Kolmer and Matsunami were able to determine the varying degrees of susceptibility to infection by the meningococcus in human beings by this same method.

They were able to measure the gradual increase in the degree of immunity to pneumococcus infection during convalescence from pneumonia.

Many investigations have been made by various workers and some of the more important bacteria, from an etiologic standpoint, were studied and their reaction to whole blood determined.

These investigations have opened up a new avenue of approach to the study of focal infections as a causative factor in the production of disease, and have revealed a new field for study by the scientific investigator and the clinician, which if developed sufficiently, may prove of inestimable value in treatment and prophylaxis. It is obvious that the problem for the clinician is to determine where the foci of infection is located; it remains for the investigator *to determine what relation this focus of infection bears to the* disease from which the patient is suffering.

The technic of this method has been fully outlined by Heist, Kolmer and Cohen, and I feel that a detailed description would be unnecessary at this time. Heist in elaborating this test made use of the many stemmed pipette of Wright, and availed himself of the suggestion of Prof. Lacy in regard to

*From the medical clinic of Dr. George Morris Piersol, and the laboratories of the Graduate School of Medicine, Dr. John A. Kolmer, director, of the University of Pennsylvania.
Read by invitation at the Fourth Annual Meeting of the American Society of Clinical Pathologists at Philadelphia, May 20-23, 1925.

the principle of capillary attraction in filling the tubes, first with the bacterial culture, then after the removal of the excess of bacterial suspension (sufficient number of bacteria adhering to wall of pipette), the same principle is used in filling the tubes with patient's blood.

The ordinary bacteriologic laboratory technic and care are used in procuring pure, uncontaminated cultures, incubation, staining, etc., to identify the various organisms.

The real point of interest as the end-result of the test is the survival of organisms after the blood is added, and the dilution of the culture which the blood has not destroyed.

Suffice it to say that a careful technic and close observance of the details of the test, are necessary if data of value are to be accumulated.

This method, as is well known, requires the isolation in pure culture of all organisms present. The bactericidal action of the whole blood of the patient is now determined against five dilutions of each organism in a series of capillary tubes, the results being evaluated according to the greatest number of organisms inhibited or destroyed by an equal volume of blood.

In order to further study the method of Heist and the Solis-Cohen in its clinical application and its therapeutic effect, I began the study of a series of cases.

This work was carried out in the laboratory of clinical pathology of the Graduate School of Medicine of the University of Pennsylvania, under the direction of John A. Kolmer.

The cases studied were selected from the dental clinic of David Goodfriend at the Polyclinic hospital, and from cases in the wards of the same hospital and from the service of G. M. Piersol.

They were all cases of focal infection presenting symptoms of many diseases, such as arthritis, corditis, neuritis, frequent sore throats, etc.

Method of Study.—A culture was taken from the infected area by means of a sterile cotton applicator, and streaked upon blood agar plates.

The original streaking was branched by making short lateral strokes across the main streaking so that the colonies would grow separately. In this way one would be enabled to more easily and accurately identify and pick out the colonies of the various organisms, thus procuring a pure culture of each.

The blood agar plates were inoculated for twenty-four hours.

The colonies were then examined both macroscopically and microscopically by smear and stain to identify the various organisms, and when necessary, cultural methods were resorted to in order to make identification positive in the event of existing question in identifying any particular organism.

A pure culture of each organism was procured and bouillon tubes inoculated. Rosenow's media was exclusively used for pure cultures of streptococcus because this media seemed by far the preferable one for this organism. The bouillon tubes and tubes of Rosenow's media were then incubated for twenty-four hours. At the end of the period of incubation the proper dilutions were made for each organism, the many stemmed pipette being used as previously suggested by Heist.

From the organisms which were not destroyed by the patient's blood an autogenous vaccine was prepared in the usual way. I shall report some of the cases studied and a brief history, result of treatment, etc.

CASE 1.—Mrs. V. S., age fifty-seven years, a housekeeper. She has had a generalized arthritis for seven years, with arms, back, and lower limbs stiff, and unable to flex limbs or move any joints of arms, hands or lower limbs. Her hands were swollen, stiff and painful; fingers stiff and swollen. She was unable to turn in bed or to help herself in any way.

Examination showed many infected teeth with apical abscesses. Teeth were extracted, and smear taken and cultured upon blood agar plates, etc.

There were isolated from the culture a pure streptococcus, Staphylococcus aureus and pneumococcus.

A vaccine was prepared from the Staphylococcus aureus, this being the only organism which the patient's blood had not destroyed at the end of twenty-four hours.

Marked and continued improvement resulted from the administration of the vaccine.

CASE 2.—N. D., male, aged fifteen years. The patient had mitral incompetency and mitral stenosis resulting from an attack of endocarditis. The teeth were in poor condition; many apical abscesses and infection about the teeth.

The diseased teeth were extracted. A culture from the teeth which were extracted showed Streptococcus hemoliticus and Staphylococcus aureus.

Upon patient's whole blood there was found surviving the twenty-four-hour incubation period the hemolytic streptococcus, but the Staphylococcus aureus had disappeared.

A vaccine was prepared from the Streptococcus hemoliticus.

No improvement resulted.

CASE 3.—S. S., male, aged twenty-one years, has had enlarged tonsils and frequent attacks of sore throat during the winter for several years. Three years ago the patient developed an otitis media in the left ear, and the drum was perforated. Following the attack of middle ear infection he had his tonsils removed. Since removal of the tonsils three years ago, he has suffered from frequent attacks of pharyngitis accompanied by an offensive breath. During each attack of pharyngitis the lymphatic glands on both sides at the angle of the jaw became enlarged His condition remained unchanged despite energetic treatment by the usual clinical methods.

A smear and culture from the rhinopharynx showed Streptococcus viridans, Staphylococcus aureus, and Micrococcus tetragenus Cultures were made upon whole blood, which showed a growth of Streptococcus viridans, but no Staphylococcus aureus or Micrococcus tetragenus.

A vaccine was prepared from the Streptococcus viridans and administered. Since then the patient has had no further recurrence of the pharyngitis.

CASE 4.—A girl, aged sixteen years, came to the clinic complaining of generalized joint involvement. She had a mitral lesion and convulsive seizures, probably epileptic. Her teeth were in bad condition. Three were extracted because of apical abscesses, and a smear from these developed a growth of hemolytic streptococcus, Staphylococcus aureus and pneumococcus.

On patient's blood only the streptococcus survived, from which a vaccine was prepared.

No improvement followed administration of the vaccine.

CASE 5.—E. C., female, aged thirty-eight years, had suffered from neuritis and generalized joint pains for three years, but no stiffness of joints was ever noted by the patient. The x-ray disclosed many dead and infected teeth. These were extracted. A smear taken from the root of an extracted tooth showed a pure culture of Streptococcus viridans, but no other organisms were found

On patient's blood the Streptococcus viridans grew and from it a vaccine was prepared.

The patient's condition was much improved by use of the vaccine.

Case 6.—N. D., male, aged twenty-six years, had suffered from attacks of rheumatism for seven years, but had no stiffness of joints or contractures. He has mitral regurgitation. At present, his joints are painful and tender. The teeth were examined and several apical foci located. These teeth were extracted, and a smear and culture made which showed a growth of nonhemolitic streptococcus and Staphylococcus aureus.

Upon patient's blood there was a growth of streptococcus, but the staphylococcus failed to grow.

A vaccine was prepared from the streptococcus.

Patient steadily improving since vaccine administration was instituted.

Case 7.—Mrs. L. W., age forty-six years, complained of pains in joints, muscular stiffness, and soreness. She has had frequent attacks of acute rheumatic arthritis which confined her to bed for variable lengths of time on various occasions.

Teeth examined and many apical foci detected.

Diseased teeth extracted. Culture showed only a growth of hemolytic streptococci, which grew upon patient's blood. A vaccine was prepared from the streptococcus and administered. No improvement resulted.

At present the patient is being treated in the clinic for a cholecystitis which may be an etiologic factor in causation of continuation of symptoms.

Case 8.—R. P., colored, male, age thirty-five years, has had frequent attacks of rheumatism for ten years. During the last four months he has been incapacitated for work because of pain in his back and joints.

Several abscessed teeth were found by x-ray. These were extracted and smears taken. Culture showed growth of streptococcus and Staphylococcus aureus.

On patient's blood the streptococcus grew well, but no growth of Staphylococcus aureus appeared.

A vaccine was prepared from the streptococcus, and patient has steadily improved since beginning its use.

Case 9.—H. N., male aged twenty-five years, came to the clinic complaining of pains in the joints, muscular soreness, and pain in the lumbar muscles. He has had these acute attacks during the winter months for the past three years. Apical foci of infection detected and the diseased teeth were extracted. A smear was taken and upon culture showed a growth of the Streptococcus viridans and a few Staphylococcus aureus.

Upon the blood only the Streptococcus viridans grew, and from this organism a vaccine was prepared.

No improvement has been noted in the patient's condition so far.

Case 10.—L. G., male, aged thirty-three years, had myocarditis following influenza. He has had frequent attacks of sore throat during the winter months for five years. A culture from the tonsil and rhinopharynx showed Streptococcus viridans and Staphylococcus aureus.

On patient's blood only the Streptococcus viridans grew, and from this a vaccine was prepared.

Patient has steadily improved since beginning vaccine treatment.

No specific conclusions can be drawn as to the therapeutic efficiency of this method from the limited number of cases that have been studied. As previously stated elsewhere, a comparison of results in a larger series of cases is necessary in order to arrive at any definite conclusions.

It was found not only from the above cited cases, but in numerous cases not cited here (because for various reasons they could not be followed up), that in nearly all cases where the streptococcus was isolated, this organism grew upon the patient's blood. This occurred in nine of the above cases,

while in only one case did it grow upon culture and not upon the patient's blood, and this was probably due to a fault in the technic.

The findings in the unreported cases studied in the laboratory also warrant this statement.

If the streptococcus is the causative agent in these cases, then it must produce the multiplicity of symptoms by its toxins. For, clinically, we know that a streptococcus bacteriemia is a more serious condition than was encountered in our cases. Again, it is difficult to believe that the nondestructive lesions of rheumatic arthritis and the destructive lesions of Streptococcus endocarditis are caused by the same organism.

If this organism were free in the blood stream in both cases, how could it produce such divergent lesions?

At present, we do not know the immediate cause of the joint symptoms in rheumatic arthritis, but since the function of the joint is not destroyed, it is difficult to attribute the lesion to the streptococcus. We would be more inclined to attribute the lesions to some bacterial toxin than to the bacteria themselves. The toxins may be absorbed from a local foci, or from a toxin derived from the destruction by the blood of bacteria reaching the blood stream from such local foci of infection, and the continued invasion of the blood stream by bacteria or their products should and probably does, increase the patient's resistance to such bacteria.

If this is true, we would expect the blood to possess sufficient bactericidal power to prevent the growth of such organisms in it. On the other hand, in a foci of infection we often isolate the streptococcus with other organisms and the streptococcus grows upon patient's blood, while the others do not.

May we not well reason that the organisms which have been reaching the blood stream have caused the patient to develop such a degree of immunity against this organism that his blood is bactericidal to it; and that the organism growing on the blood is harmless in so far as its having been entering the blood stream is concerned?

Consequently the bactericidal power of the blood has not developed against this specific organism.

CONCLUSIONS

From observation and study of this problem it is believed that the following conclusions are at present justified.

1. The whole coagulable blood of man may possess the property of destroying or inhibiting the growth of certain pathogenic bacteria.

2. Bacteria which will not grow in the whole blood in vitro but are destroyed by it, are probably incapable of producing a bacteriemia.

3. Bacteria which will grow in the whole coagulable blood in vitro are probably capable, if they reach the blood stream, of producing a bacteriemia.

4. Bacteria which will not grow in the whole coagulable blood of a patient in vitro may still produce a localized infection with the production of symptoms due to toxins and other bacterial products absorbed from such lesions with the production of symptoms.

5. Bacteria from a local infection capable of growing in the patient's blood in vitro may not be the cause of the disease, although potentially capa-

ble of infecting the blood, until this tissue acquires bactericidal properties by immunologic processes.

6. Autogenous vaccines prepared of bacteria from localized infections weeded out by this method are not necessarily specific although therapeutic results may be secured by nonspecific protein reactions.

7. Until further study has been made and more data accumulated, this method is worthy of further study and especially in relation to the perplexing problem of deciding which organism in a mixed localized infection should be incorporated into autogenous vaccines.

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THE VALUE OF ACETONE DETERMINATION IN EXPIRED AIR IN CORRELATION WITH ALVEOLAR CARBON DIOXIDE TENSION*

Practical Methods, Technic, and Apparatus

By Paul Roth, M D , Battle Creek, Mich.

THE value of carbon dioxide determinations in alveolar air and a simple test for acetone in the expired air were brought to my attention during a period of study of the respiratory exchange and methods of estimation at the Carnegie Nutrition Laboratory, in Boston, in 1912 and 1913. H. L. Higgins, then a member of the staff of that institution, was carrying on, under the direction of F. G. Benedict, various observations in connection with extensive experimental studies being made there on diabetes. The laboratory procedures used and perfected by Higgins[1] promised to be of clinical value and were at once introduced at the Battle Creek Sanitarium for purposes of diagnosis and as therapeutic guides in diabetes. They soon gained the favor of members of the staff as a means of detecting and measuring the degree of acidosis, not only in diabetes but in various other conditions. At the request, especially of C. E. Stewart, then chief of the diabetic service, these tests were about a year later included in the list of routine examinations to which all the patients are subjected and methods then used[2] were later modified[3]; the latest improvements being now published for the first time.

The preference is still given to these gasometric methods because they are as yet the only ones which are simple enough and sufficiently reliable to be practical for the wide and frequent use to which they are put. Direct observations upon the blood by the methods of Van Slyke[4]; Cullen[5]; Myers, Schmitz and Booher[6] are resorted to only in selected cases. A test for acetone in the expired air has been from the start routinely done simultaneously with the determination of the alveolar CO_2 tension.

During the first few years after the introduction of these tests, at a time when the management of diabetes was far from being as efficient as it is today, severe acidosis was frequently observed and fatal diabetic coma was not of rare occurrence

The clinical application of these tests further showed that acetone occurs very frequently in disease and readily appears even in normal individuals from as slight a provocation as missing a meal or two. Needless to say that the presence of acetone has the same significance whether found in the expired air, where it is most easily detected, or in the blood or urine. It is invariably associated with acidosis of ketonic origin. Its absence when acidosis is present, therefore, indicates that the acidosis is due to entirely different

*From the Metabolism Laboratory of the Battle Creek Sanitarium, Battle Creek, Mich. Read before the Fourth Annual Convention of the American Society of Clinical Pathologists, in Philadelphia, May 20-23, 1925

causes such as those associated with or due to renal insufficiency. This is the so-called "phosphatic type" of acidosis. These two types of acidosis often coexist; therefore, the occurrence of acetone does not rule out the possibility of a phosphatic type of acidosis being also present.

Acidosis can readily be detected by the various simple and well-known procedures which are to be found in any up-to-date textbook of physiology and of physiologic chemistry. It so frequently occurs in disease that the clinician will find it well worth while to systematically look for it in both acute and chronic affections. The dangers of severe acidosis are in general fully recognized, but when it is of moderate intensity its significance and

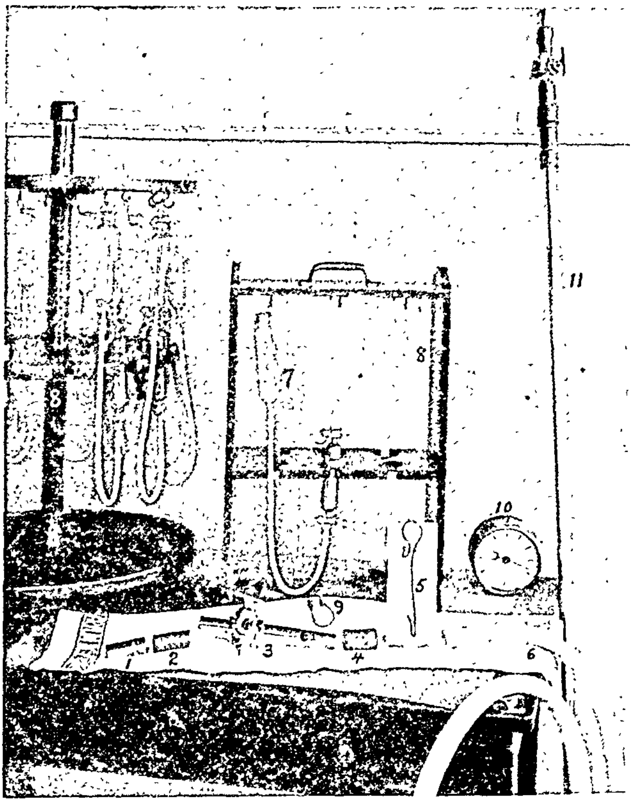

Fig. 1.—Equipment for obtaining a sample of alveolar air by the author's method. Disassembled parts from a previous paper[3] which gives a detailed description of each of the numbered parts. A ⅜ inch three-way cock is now used.

bearing should not be lost sight of. The clinician who is interested in the border line of disease and in preventive medicine will find this field of investigation of fundamental importance.

The normal metabolic processes throughout the body are dependent upon remarkably close fundamental biologic conditions. Even a slight departure from these means disaster which is efficiently averted by delicate and complex mechanisms. One of these mechanisms is that which regulates the reaction or ionization of the body fluids.[7] Whenever we find that such a mechanism has become persistently inadequate, the danger signal should be heeded, because any departure from the normal is the stepping-stone for

metabolic disturbances of various sorts. Vital energy is depressed, repair and recovery are retarded, senescence is hastened,—in fact, both acidosis and alkalosis are incompatible with life and health. Subjects with acidosis, as is well known, are poor surgical risks. In an unpublished review of the alveolar CO_2 tension in 260 consecutive major surgical operations performed at the Battle Creek Sanitarium, eight to ten years ago, 174 (67 per cent) showed a CO_2 tension below the minimum normal before operation. While the death rate, less than twenty days after the operation, amounted to 11 per cent of these cases, there was a mortality of only 0.5 per cent among those who showed a normal CO_2 tension before the operation. These figures at least show that acidosis adds a material risk in surgical cases.

The frequency of acidosis in general clinical cases was well shown in a simultaneous review of 2,305 consecutive cases. A conservative estimate

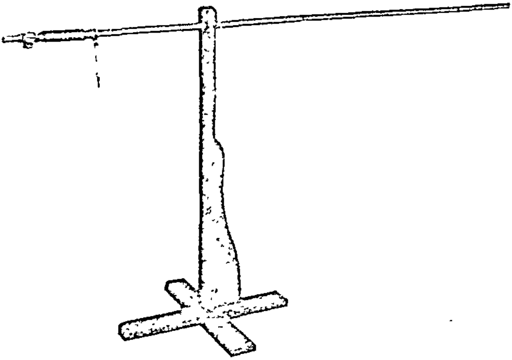

Fig. 2.—Collecting tube (5 ft. long, ¾ inch, capacity about 500 c.c.) with support.

showed that 44 per cent of these cases gave an alveolar CO_2 tension below 37 mm. Severe acidosis was observed in about 10 per cent of the total number.

Alkalosis is of late attracting the attention that it deserves and much of what has just been said about acidosis applies also to alkalosis. This shifting of the "acid-base" balance toward the alkaline side is very ably discussed in a recent paper by Kast, Myers and Schmitz.[8] With this same paper a very well selected list of references to the literature on the subject is given.

The present study was undertaken with the full expectation that a careful investigation of a large number of cases would confirm the observations casually made a few years ago. The diagnosis and the laboratory findings of one thousand cases, taken at random from the records of the past three years, were tabulated. In every case the diagnosis was confirmed by urinalysis, blood chemistry, alveolar CO_2 tension, and acetone in the expired air. These tests were performed on the same day with but few exceptions,

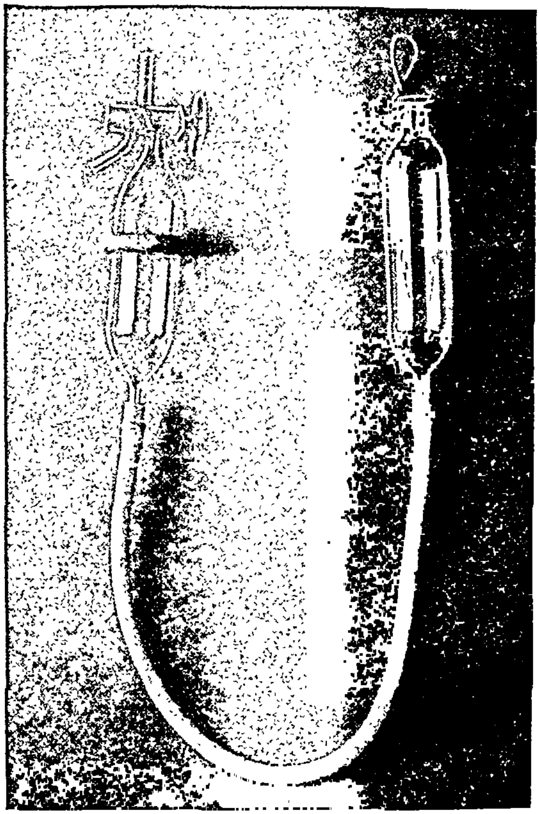

Fig. 3.—Collecting burette unit.

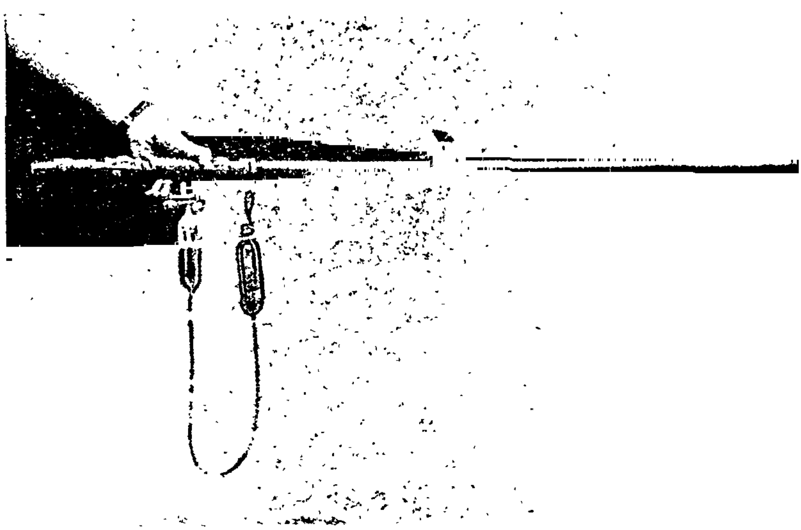

Fig. 4.—Collecting tube with burette in place. The burette is filled with mercury to the very tip, which is inserted through a small opening in the rubber sleeve.

and then none over three days apart. The accompanying table presents the most striking points observed which fall within the scope of this paper. The cases were classed under the following heads:

1. *Diabetics (210 cases).*—In this group were placed only those diabetics who did not show any renal involvement; no albumin, no casts in the urine, a blood nonprotein nitrogen not over 34.0 and blood uric acid not over 5 mg. per 100 c.c. A very few showing an amount of uric acid above the normal without any other evidence of renal insufficiency were also included in this group. These diabetics were all of the moderate type.

2. *Diabetic-Renal (221 cases)*—Representing all degrees of diabetes with definite signs of renal involvement.

3. *Renal (242 cases).*—Including all cases with renal involvement without any signs of diabetes.

Fig. 5.—Collecting alveolar air after 20 seconds, rebreathing through the 5 foot tube. The nose should be clamped.

4. *Others (500 cases).*—Showing no renal involvement or any diabetic taint.

Each class is divided into two groups.

A. *No Acetone.*—Cases having shown no acetone in the expired air either at the entrance examination test or in any subsequent tests.

B. *With Acetone.*—Cases having shown acetone in the expired air one or more times.

The alveolar CO_2 tension recorded under each group is divided into four ranges:

1. The Normal,	with	tensions of	40 mm. or over.
2. Mild acidosis	"	"	35 to 39 mm.
3. Moderate "	"	"	30 to 34 mm.
4. Severe "	"	"	29 mm. or less.

TABLE I

THE ALVEOLAR CO_2 TENSION IN THE PRESENCE OR ABSENCE OF ACETONE IN EXPIRED AIR

(1000 cases, years 1923 and 1924.)

Their relative occurrence in: (1) Diabetes without renal involvement (2) Diabetes with renal involvement (3) Renal cases without diabetes (4) Other cases with no renal or diabetic symptoms.

ALV. CO_2 TENSION MM.	1. DIABETES		2. DIABETIC-RENAL		3. RENAL		4. OTHERS	
	Without Acetone							
	36%		40%		69%		71%	
	Cases	%	Cases	%	Cases	%	Cases	%
40 - 50	15	33	23	36	54	37	121	37
35 - 39	28	61	32	50	72	49	181	55
30 - 34	3	6	9	14	17	12	24	7
29 or less	0	0	0	0	3	2	4	1
	With Acetone							
	64%		60%		31%		29%	
40 - 50	25	31	27	28	20	31	52	31
35 - 39	47	57	59	61	30	46	94	55
30 - 34	10	12	9	9	11	17	23	13.5
29 or less	0	0	2	2	4	6	1	.5

Tensions between 30 and 39 mm. are included under two separate ranges merely because of the great difference of frequency observed in these two ranges.

The chief facts which this table shows are as follows:

1. Acetone occurs in the expired air twice as frequently in diabetics (64 per cent) and in the diabetic-renal (60 per cent) as in exclusively renal (31 per cent) and "other" cases (29 per cent).

2. The relative number of cases with a normal CO_2 tension is practically the same in all four groups whether acetone is present or not.

3. Mild acidosis is slightly more frequent in the diabetics (59 per cent) and diabetic-renal (56 per cent) than in the renal (48 per cent) and other cases (55 per cent).

4. Moderate acidosis is slightly more frequent in renal cases (13 per cent) and in diabetic-renal (11 per cent) than in diabetics (10 per cent) and other cases (9.5 per cent).

5. Severe acidosis occurred more frequently in renal cases (3.5 per cent) and in other cases (1 per cent) than in diabetics (only 0.7 per cent). (In a series of 36 diabetics examined previous to 1920, severe acidosis occurred in 11 per cent of the cases.)

6. Severe acidosis was not observed in the group of 128 consecutive cases of diabetes whether acetone was present or not.

7. Severe acidosis was likewise not found in the group of 146 diabetic-renal cases without acetone.

8. Severe acidosis occurred in only 2 per cent of the diabetic-renal cases with acetone. (In a series of 40 diabetic-renal cases with acetone examined previous to 1920, severe acidosis occurred in 7.5 per cent of the cases.)

The reader will find in the references to the literature given here, complete descriptions of the methods referred to in this paper.

Figs. 1 to 8 present my modifications and improved methods for collect-

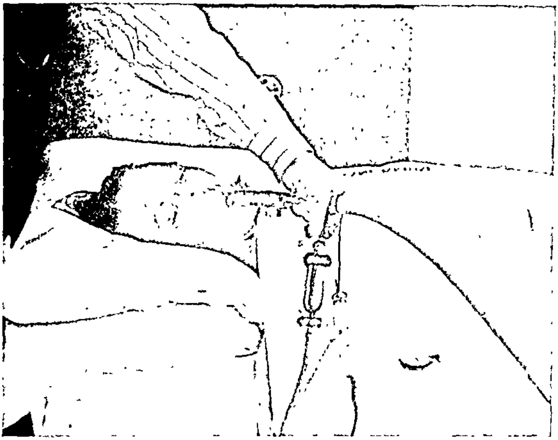

Fig. 6.—Collecting alveolar air (as in Fig. 5) using a 5 foot ¾ inch rubber tube. Subject in bed.

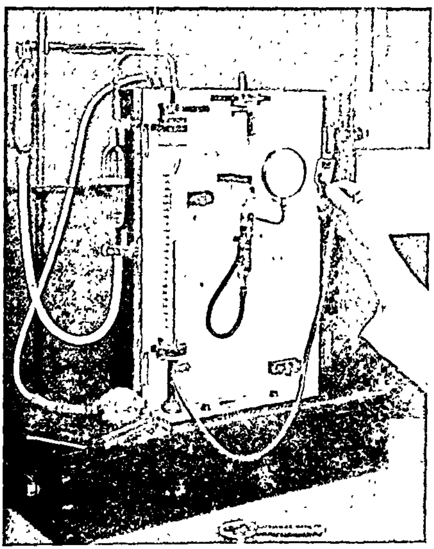

Fig. 7.—A Haldane Air Analysis Apparatus modified for CO_2 analysis only. Sampling burette in place.

ing alveolar air and for the determination of the CO_2 tension by analysis of the sample with the Haldane[9] or Henderson[10] apparatus.

Figs. 8 to 16 illustrate the application of Marriott's method for alveolar CO_2 tension determination.[11] Although my method is generally used on account of being both speedier and of greater accuracy, Marriott's method has for years been found to be exceedingly satisfactory and most reliable for bedside use. The results obtained by this method have systematically been compared and the solutions and standards checked by repeated analyses for the past several years. The results checked usually within 1 or 2 per cent; a

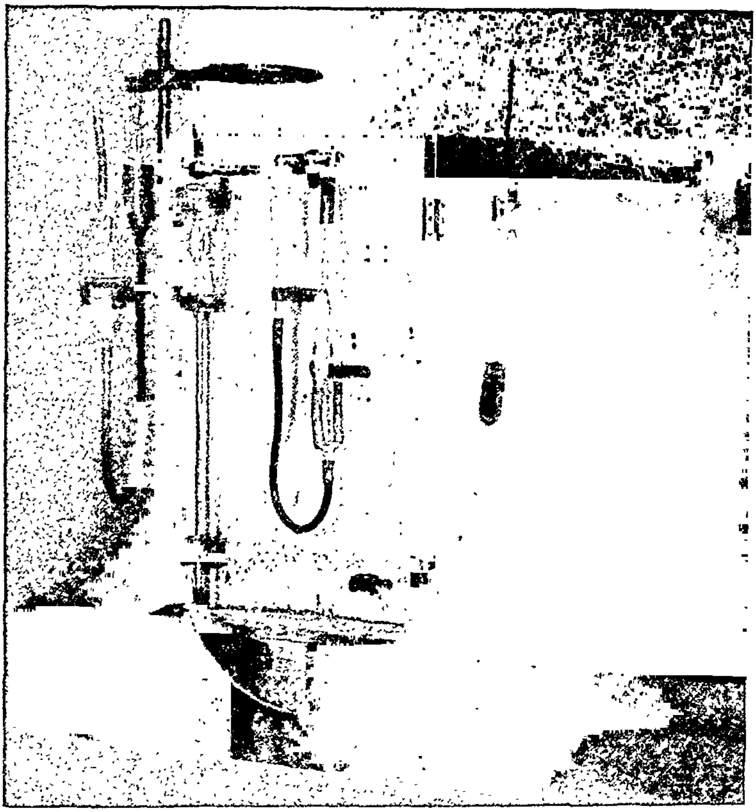

Fig. 8.—Same as Fig. 7 with a shortened water jacket intended to protect the bulbs of the burettes only. The graduated stem is thus better exposed for reading.

difference of 3 per cent being rarely observed. The standards are now very permanent. I am informed by the makers that a suitable mask and a three-way cock will be furnished with the outfit on request.

The football bladder with an enlarged opening (Fig. 11) presents the advantage of being self-inflating and does not require the use of a hand bulb.

Figs. 17 and 18 show my simple tube for the detection of acetone in the expired air using Scott-Wilson solution.

Test for Acetone in Expired Air.—The subject is asked to take a moderate breath and to expel it completely through 2 or 3 c.c. of Scott-Wilson solution (see below) placed in a test tube or in my apparatus (Fig. 18). The test is reported negative if the solution remains perfectly clear after three or

four moderate breaths have been expelled through the solution. A pearly opalescent clouding or precipitate is due to acetone, the amount of which is expressed as trace, plus, double plus, etc.

Scott-Wilson solution[12].—To 10 gm. of mercuric cyanide dissolved in 600 c.c. of water add a cooled solution of 180 gm. of sodium hydroxide in 600 c.c. of water. Transfer this mixture to a heavy walled jar, and to it add 2.9 gm. of silver nitrate dissolved in 400 c.c. of water. The silver solution should be added in a slow stream, and the addition must be accompanied by constant and vigorous stirring with a glass rod. If properly made, the silver dissolves

Fig. 9.—Outfit for the collection of alveolar air and the determination of the CO_2 tension (Marriott Method) and acetone in the expired air (Roth).

completely, giving a clear solution which is at once available for use. If the solution is turbid, it should be set aside to settle for three or four days and the clear supernatant liquid removed by means of a siphon.

In the clear reagent a new sediment gradually forms, so that the solution deteriorates slowly, and after a few months is not serviceable for quantitative work, though still good for qualitative tests.

DISCUSSION

It must be borne in mind that the diabetics and other cases treated at the Battle Creek Sanitarium are not likely to come from a medically neglected

class of patients. On the whole they are more likely to have had, before coming to this institution, better and more modern medical care than is generally found. Therefore, the occurrence of severe acidosis would unques-

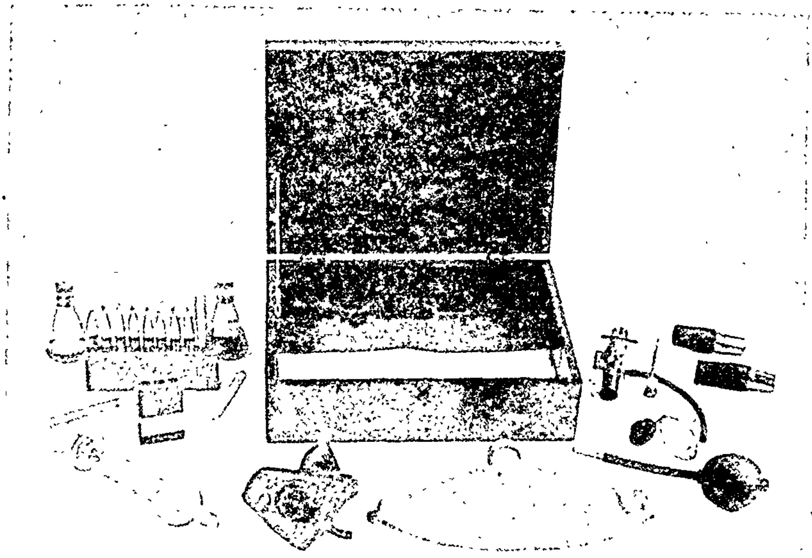

Fig. 10.—Marriott alveolar CO_2 tension outfit, as modified by the writer.

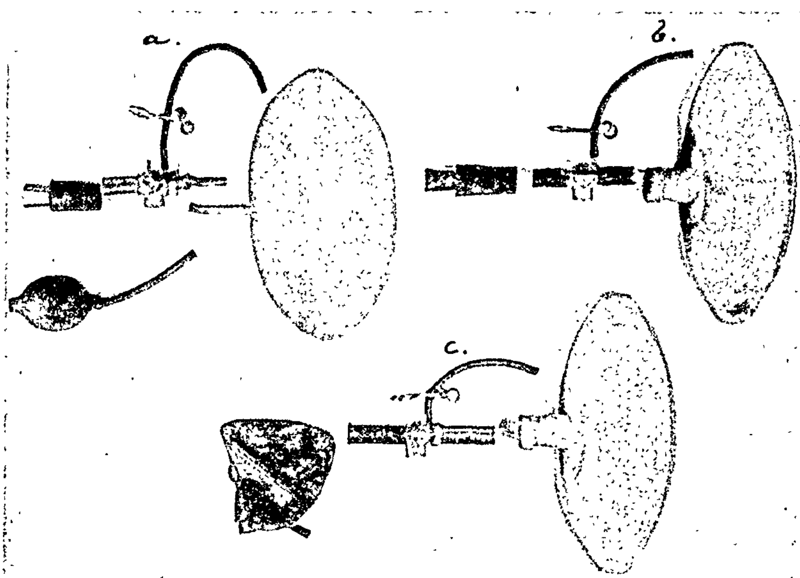

Fig. 11.—Three types of collecting units. (*a*) Using an ordinary football bladder. Requires a rubber bulb for inflation. (*b*) Bladder with ¾ inch opening. Practically self inflating. (*c*) Same with mask in place of mouthpiece.

tionably be relatively lower than in cases generally admitted in hospitals. Severe acidosis is nowadays very rarely encountered in the class of cases referred to above. There is very little question about this being the result of a general better understanding of the proper treatment of this disease. The

use of insulin is no doubt bearing splendid results but is not wholly responsible for them. No clinician can continue to remain unacquainted with the best and indispensable dietetic measures the moment the use of insulin

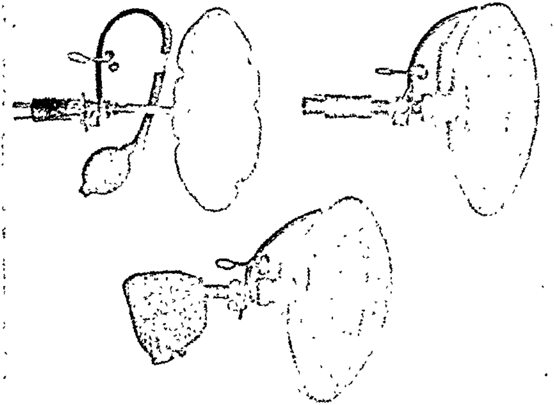

Fig 12—Same as Fig 11, collecting units assembled.

Fig. 13.—Collecting alveolar air for CO_2 tension determination, using a mouthpiece

is considered. With the high fat and more especially with the high protein diet generally advocated in diabetes until quite recently, acidosis was strongly encouraged and difficult if not impossible to control.

C. C. Hubly, who for a number of years has been chief of the diabetic service and through whose kindness I have had access to the records of his most efficient work, informs me that the number of undiagnosed cases of diabetes has considerably decreased in recent years. Acidosis is more suc-

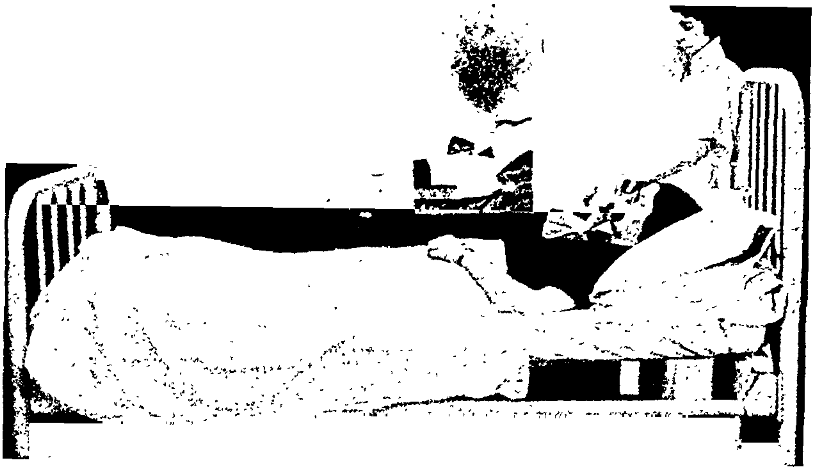

Fig. 14.—Collecting alveolar air for CO_2 tension determination, using a mask.

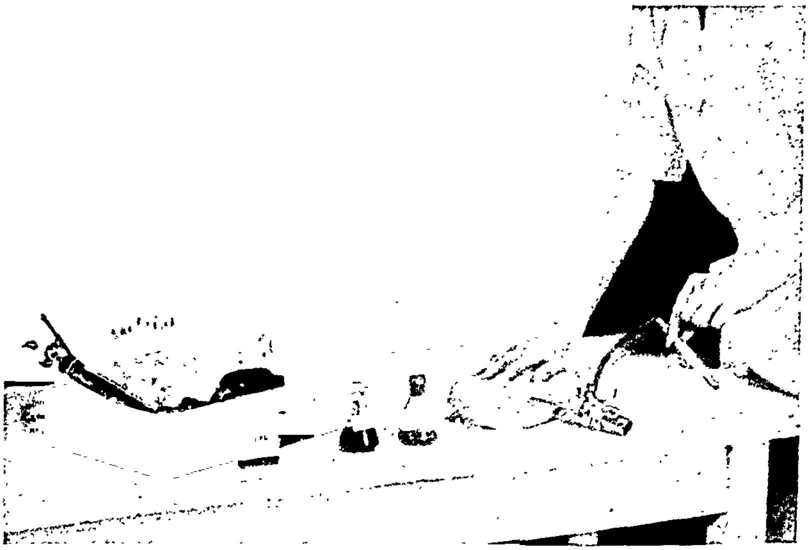

Fig. 15.—Expelling alveolar air and bubbling it through the standard bicarbonate solution with indicator "phenolsulphonephthalein" in test tube. (Marriott.)

cessfully treated when it is of the ketonic type alone and it is indicated by the presence of acetone in the urine or in the expired air. Proper dietetic and other therapeutic measures very quickly cause a disappearance of the acetone and a return of the alveolar CO_2 tension to the normal in the cases uncomplicated with renal involvement. The disappearance of acetone with a CO_2 tension below the normal must mean that while the ketonic acidosis is completely

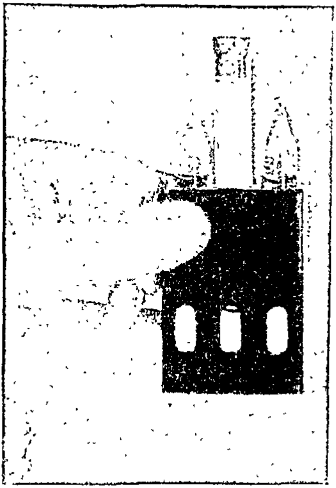

Fig. 16.—Colorimetric estimation of CO_2 tension. Comparing the unknown with marked standards (Marriott.)

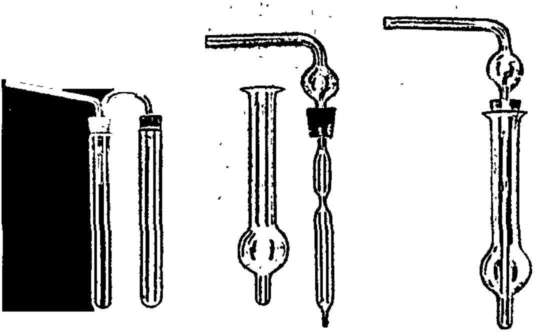

Fig. 17.—Left, test tube unit for detecting acetone in the expired air Center, safety detector of acetone in expired air (Roth). Right, the same assembled. The tip end of the external tube only is filled with "Scott-Wilson" solution

held in check, an acidosis due to renal insufficiency is still present. It seems that an acidosis of renal origin is more difficult to control and to eradicate. This is shown by the fact that not infrequently the well-managed cases of diabetes can readily be kept acetone-free in spite of the persistence of a more

Fig 18.—Safety acetone detector, as used. (Roth.)

or less pronounced acidosis. The treatment of this type of acidosis is as yet poorly understood.

I am greatly indebted to my colleagues, C. C. Hubly and W. B. Lewis, chief chemist, for their helpful cooperation and suggestions in this study.

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(For discussion, see page 295.)

STUDIES IN LOCAL ANESTHESIA. III*

THE PHARMACOLOGY OF SOME PARA-AMINO-BENZOATE COMPOUNDS

Türck's Reflex Method in the Determination of Local Anesthesia

BY W. J. R. HEINEKAMP, M.S., M.D., CHICAGO, ILL.

THE method used is based on Turck's method of recording reflex time. The frog's leg is placed about midway to the knee in a solution of the anesthetic for ten consecutive minutes and the effect determined at definite intervals by immersing the foot in tenth-normal HCl. If anesthesia was produced, the foot was not withdrawn from the acid solution. If anesthesia had not been produced, the foot was withdrawn.

Protocol 1.—

1 per cent Cocaine Hydrochloride

Frog		
I	Right leg	10 min.
	Left leg	10 min.
II	Right leg	10 min.
	Left leg	10 min.
III	Right leg	10 min.
	Left leg	10 min.

Protocol 2.—

A. I.

Frog		
I	Right leg	No anesthesia 1 hr.
	Left leg	" " "
II	Right leg	" " "
	Left leg	" " "
III	Right leg	" " "
	Left leg	" " "

Protocol 3.—

A. II.

Frog		
I	Right leg	No anesthesia 1 hr.
	Left leg	" " "
II	Right leg	" " "
	Left leg	" " "
III	Right leg	" " "
	Left leg	" " "

Protocol 4.—

A. III.

Frog		Anesthesia
I	Right leg	10 min.
	Left leg	10 min.
II	Right leg	10 min.
	Left leg	10 min.
III	Right leg	10 min.
	Left leg	10 min.
		Average 10 min.

*From the University of Illinois, College of Medicine, Chicago.

Protocol 5.—

A. IV.

Frog		Anesthesia
I	Right leg	15 min.
	Left leg	15 min.
II	Right leg	10 min.
	Left leg	10 min.
III	Right leg	10 min.
	Left leg	15 min.
		Average 12.5 min.

Protocol 6.—

A. V.

Frog		Anesthesia
I	Right leg	30 min.
	Left leg	35 min.
II	Right leg	30 min.
	Left leg	20 min.
III	Right leg	30 min.
	Left leg	20 min.
		Average 27.5 min.

Protocol 7.—

A. VI.

Frog		Anesthesia
I	Right leg	30 min.
	Left leg	50 min.
II	Right leg	30 min.
	Left leg	40 min.
III	Right leg	30 min.
	Left leg	30 min.
		Average 35 min.

Protocol 8.—

A. VII.

Frog		Anesthesia
I	Right leg	15 min.
	Left leg	20 min.
II	Right leg	25 min.
	Left leg	15 min.
III	Right leg	15 min.
	Left leg	15 min.
		Average 17.5 min.

Protocol 9.—

B. I.

Frog		
I	Right leg	No anesthesia 1 hr.
	Left leg	" " "
II	Right leg	" " "
	Left leg	" " "
III	Right leg	" " "
	Left leg	" " "

Reflex active at end of hour.

Protocol 10.—

B. II.

Frog	Conditions as with Cocaine.	
I	Right leg	10 min.
	Left leg	15 min.
II	Right leg	20 min.
	Left leg	20 min.
III	Right leg	15 min.
	Left leg	20 min.
		Average 15 min.

Protocol 11.—
B. III.

Frog.	Milky fluid.	
I	Anesthesia	60 min.
II	Anesthesia	50 min.
III	Anesthesia	50 min.
	Reflex brisk at 1 hour.	

Protocol 12.—
B. IV.

Frog		
I	Right leg	10 min.
	Left leg	10 min.
II	Right leg	10 min.
	Left leg	10 min.
III	Right leg	10 min.
	Left leg	10 min.
	Average	10 min.
	No anesthesia at 5 min.	

Protocol 13.—
B. V.

Frog		
I	Right leg	10 min.
	Left leg	15 min.
II	Right leg	15 min.
	Left leg	10 min.
III	Right leg	10 min.
	Left leg	10 min.
	Average	11.6 min.

Protocol 14.—
B. VI.

Frog		
I	Right leg	15 min.
	Left leg	10 min.
II	Right leg	10 min.
	Left leg	10 min.
III	Right leg	10 min.
	Left leg	15 min.
	Average	11.6 min.

Tables I and II summarize the protocols.

TABLE I

NO.	NAME	ONSET OF ANESTHESIA
	Cocaine 1%	10 min.
	Novocaine 1%	No anesthesia in 1 hr.
A. 1	Di-methylamino methyl para-amino-benzoate	" " " "
A. 2	Di-ethylamino ethyl para-amino-benzoate	" " " "
A. 3	Di-normal propylamino ethyl para-amino-benzoate	10 min.
A. 4	Di-normal butylamino ethyl para-amino-benzoate	12.5 "
A. 5	Di-isopropylamino ethyl para-amino-benzoate	27.5 "
A. 6	Di-isobutylamino ethyl para-amino-benzoate	35 "
A. 7	Di-secondary butylamino ethyl para-amino-benzoate	17.5 "
B. 1	Di-ethylamino propyl para-amino-benzoate	No anesthesia in 1 hr.
B. 2	Di-isopropylamino propyl para-amino-benzoate	15 min.
B. 3	Di-nor.-propylamino propyl para-amino-benzoate	53.3 "
B. 4	Di-nor.-butylamino propyl para-amino-benzoate	10 "
B. 5	Di-isosecondary butylamino propyl para-amino-benzoate	11.6 "
B. 6	Di-isobutylamino propyl para-amino-benzoate	11.6 "

Designating ten minutes the time necessary for 1 per cent cocaine HCl to totally inhibit a reflex with tenth-normal HCl used as a stimulus as unity, the drugs in molecular concentration corresponding to 1 per cent cocaine HCl have the following comparative values shown in Table II.

RECAPITULATION SERIES

MINIMUM TO PRODUCE ANESTHESIA

A-2	10 mg.
A-3	7 mg.
A-4	17 mg.
A-5	15 mg.
A-6	12.5 mg.
A-7	7 mg.

MINIMUM FOR ANESTHESIA

B-1	20 mg.
B-2	15 mg
B-3	18.5 mg.
B-4	2.5 mg.
B-5	17 mg.
B-6	21 mg.

CONCLUSION

This is a comparatively simple, accurate, and efficient method of standardizing the local anesthetics. When used in this way, the results coincide closely with those obtained by other methods.

This method shows that *A-3* and *B-4* are especially valuable and deserve consideration as local anesthetics for general use. They are more efficient than procaine, but slightly more toxic. Since less of the drug is required to produce the anesthetic effect, toxicity is the same as procaine with *A-3,* and less with *B-4,* which is four times as active while only twice as toxic.

COMMUNICATION

Editor, JOURNAL OF LABORATORY AND CLINICAL MEDICINE,

Dear Sir:

In the July, 1925, number of the JOURNAL OF LABORATORY AND CLINICAL MEDICINE the writer published a paper on a complement-fixation test under the title "A Modification of the Kolmer Reaction." This title is misleading and should read "A Complement-Fixation Test Using Kolmer's Antigen." This is not a modification of Doctor Kolmer's well-known technic which the writer concludes is the best of all the complement-fixation tests, but merely a system in which the Kolmer antigen is used with excellent results.

The signer trusts that this communication will eliminate any misunderstanding that may have arisen from the title of the paper.

Very truly,

(Signed) F. O. Huntsinger.

TRANSACTIONS

FOURTH ANNUAL MEETING OF THE AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS

Philadelphia, Pa., May 20, 21, 22 and 23, 1925.

DR. JESSIE W. FISHER, OF MIDDLETOWN, CONN., read a paper entitled **Autopsy Report of Two Cases of Thymic Death During Surgical Operations.** (For original article, see page 241.)

DR. B. S. PARKS, OF PARKERSBURG, WEST VIRGINIA, read a paper entitled **The Bactericidal Action of Whole Blood as Determined by the Heist-Lacy Method.** (For original article, see page 269.)

DR. PAUL ROTH, OF BATTLE CREEK, MICH., read a paper entitled **The Value of Acetone Determination in Expired Air in Correlation with Alveolar Carbon Dioxide Tension.** (For original article, see page 275.)

DISCUSSION

Dr. Frank W. Hartman.—This paper is interesting because it shows what the routine application of any laboratory procedure will pick up as compared with its application only in such cases as it seems indicated. Comparable results are found in the routine application of the Wassermann test as contrasted with its application only where it seems indicated from the history or physical examination.

Doctor Roth spoke of phosphatic acidosis. This terminology is appropriate just at this time since British workers have demonstrated large increases in the inorganic phosphates of the blood in nephritis, using these observations as a prognostic sign. In the few cases of late nephritis which we have observed, the inorganic phosphate content of the blood seems to be of more prognostic value than the creatinine content.

Dr. Paul Roth (closing).—Folin made the statement some years ago that acetone was not responsible for the fruity odor sometimes observed in the breath of diabetics.

In the last stages of severe acidosis a decrease in the amount of acetone in the urine, which sometimes is observed, is a bad prognostic sign, as it indicates that the oxidation of the ketonic acids is still further interfered with and as a result the acidosis is more severe. On the other hand, of course, the diminution or disappearance of acetone in the urine or the expired air, in the absence of acidosis, means improvement.

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EDITORIALS

Principles Relating to the Treatment of Fractures

DURING the past few years numerous articles dealing with the treatment of fractures have appeared. These seem to have demonstrated that operative treatment is not required in the majority of cases. Nonunion due to suppuration, the presence of a foreign body, or interference with normal reparative processes has given rise to a prolonged disability following operative interference in many cases quite out of proportion to that occurring in cases treated by the closed method in which the anatomic results may be regarded as imperfect.

Methods of reduction and maintenance of reduction differ, but certain principles which remain the same must be followed in the treatment of fractures. The following three principles, often neglected, cannot be repeated too often: (1) The long fragment that can be controlled must be dressed in line with the short fragment that cannot be controlled. (2) The mechanism of fractures must be understood, for in the reduction the force must be

reversed and the parts dressed in the reversed position. (3) Immobilization must be maintained for varying lengths of time, depending upon whether compact or cancellous bone or ligaments are involved.

Fractures through the surgical neck of the humerus, through the radius or both bones of the forearm between the attachment of the supinator and the pronator radii teres, and fractures of the femur below the lesser trochanter illustrate the necesity of dressing the long fragment which can be controlled in line with the short fragment which cannot be controlled.

Muscular action is an important factor in the displacement of fragments in these fractures. In fractures of the surgical neck of the humerus the upper fragment is abducted and rotated out by the supraspinatus, the infraspinatus and the teres minor. This fragment cannot be controlled, and the long lower fragment must therefore be dressed in line with it. The aeroplane splint or the old Stromeyer axillary pad answers in these cases, for the lower fragment is abducted and rotated outward somewhat. In fractures of the radius between the attachment of the supinator and the pronator radii teres, the upper fragment will be flexed somewhat by the biceps and supinated by the supinator. It is impossible to control this short upper fragment, and the lower fragment, which is long, should be dressed in supination to bring it in line with the upper, the forearm being flexed to overcome the action of the biceps on the upper fragment. The bones should be dressed in the same position in fractures of both bones of the forearm at this level. Fractures through the radius or both bones of the forearm in the lower fourth offer considerable difficulty for in these cases the pronator quadratus exerts a direct pull, and in fractures of the radius at this level it is difficult to correct the deformity even when the pronator quadratus is relaxed by full pronation. Reduction is especially difficult when both bones are fractured at this level because the pronator quadratus cannot be relaxed, a direct pull being maintained in spite of any position that the fragments can be placed in.

The displacement of the upper fragment in fractures of the femur below the lesser trochanter is typical and constant, the upper fragment being flexed, abducted and rotated outward. The lower long fragment must be dressed in line with the short upper fragment which cannot be controlled. Even when a plate is applied in this fracture the fracture must be dressed in some flexion, with abduction and external rotation. If it is not so dressed, the muscle pull may be great enough to pull out the screws. It makes little difference what kind of dressing is applied in the closed method providing longitudinal displacement of the long lower fragment is prevented and it is brought in line with the short upper one.

The mechanism of the different fractures must be understood if they are to be intelligently treated. It has been repeatedly stated that fractures of the lower end of the humerus are dressed in acute flexion because the triceps acts as a splint, and because it is much easier to extend than flex the forearm if some fixation occurs. These statements are true, but the main reason for acute flexion is that in this position the force has been reversed and the fracture is dressed in the reversed position. Probably about 95 per cent of the supracondylar fractures of the humerus are extension fractures, the line of

fracture running obliquely from above and backward downward and forward. In the extension fracture the force is reversed during the reduction and the parts dressed in the reversed position—acute flexion. Approximately 5 per cent of these fractures are extension fractures, the line of fracture passing from above and in front downward and backward. If this fracture were dressed in acute flexion, the deformity would be increased. This fracture should be dressed in extension, the force being reversed and the fracture dressed in the reversed position.

Reversal of the force—an important factor in the reduction of fractures—is especially well illustrated in Colles' and Pott's fracture. Colles' fracture is a fracture by extension combined with a twist. In reducing this fracture after the fragments are unlocked, the force is reversed, and the hand is dressed in flexion combined with some pronation of the lower fragment. The direction of the force which produced the fracture is merely reversed. The hand when the fracture is reduced falls naturally into flexion with slight pronation. It should be dressed in this position. The same principle applies to Pott's fracture, in the reduction of which the force is reversed.

Cancellous bone repairs quickly and surely. Fractures through the thick part of the neck of the femur, through cancellous bone, repair rapidly; while fractures through compact bone may heal slowly, a higher percentage of nonunion occurring than in cases in which cancellous bone is fractured. Ligamentous repair occurs slowly, and in Pott's fracture, for example, and other fractures where ligaments are involved, immobilization should be continued for a long time.

Experience increases and statistics accumulate which demonstrate the superiority of the modern closed methods, following the principles just discussed, over any operative procedure, which should only be employed when the fragments are so displaced or held by transfixed muscle or tendon that they cannot be brought into line.

David's observations on the fractures of the femur in children are of a great deal of interest and importance. He has shown that compensatory lengthening may occur and is a factor in reducing the necessity of operative interference in these cases. Alignment of fragments to prevent angulation and extension to prevent overriding are of greater importance than end-to-end apposition. Firor has shown that immobilization in plaster of Paris does not give satisfactory results in fractures of the femur in patients over fourteen years of age. The results are good in patients between seven and fourteen; and in children under seven the results are as good as by overhead extension. In adults extension should be applied—skeletal preferably—combined with a Thomas splint and overhead extension.

The principles of dressing the long fragment that can be controlled in line with the short fragment that cannot be controlled; of knowing the mechanism and reversing the force during reduction and dressing in the reversed position; and that different types of tissue repair differently, render the treatment of fractures relatively simple.

—*D. L.*

The Treatment of Paresis by Induced Fever

SYPHILIS of the brain has long been recognized as an intractable condition and one in which all that could be hoped for as a result of treatment was a symptomatic arrest, due in some measure possibly to the accepted axiom that the more specialized the tissue, the less its ability to repair or compensate for the damage consequent upon trauma or disease.

That such an involvement is greatly to be feared, the character and gravity of the symptomatology amply demonstrates; that the treatment of this type of syphilis is unsatisfactory, the long list of drugs and methods tried renders obvious.

Indeed, so well is this recognized, and so generally is it accepted that once a neurosyphilitic always a neurosyphilitic, that the major aim of the modern treatment of syphilis with its emphasis upon persistence and duration; upon repeated periodic courses of treatment; and upon life-long, periodic serologic examinations is avowedly to protect the syphilitic against and prevent the occurrence of late cerebrospinal involvement,—for we are not yet able to tell when the infection has been absolutely and finally eradicated in any case.

Among the numerous means employed for the treatment of paresis has been the injection of nonspecific substances such as milk, peptone, and other agents as adjuvants to the use of specific remedies, on the assumption of a provocative action upon the dormant foci of the disease with resultant stimulation of antibody production.

Following the observation that in a perceptible number of paretics, in whom there had been a protracted febrile illness, a remission of symptoms occurred, Wagner von Jauregg[1] conceived the idea of deliberately producing a febrile reaction, using tuberculin for the purpose, and in 1909, reported that paretics so treated lived longer and had more numerous and more protracted remissions than a parallel series not so treated.

He later used antityphoid vaccine for this purpose and, still later, observing that the best results occurred in patients having some infectious disease during the nonspecific treatment, proposed the inoculation of paretics with malaria as a pyrexial agent and reported a series of nine patients so treated two years before.[2]

In 1922, the same observer reported that a series of 200 such cases showed evident improvement, fifty having a sufficiently complete remission to enable a return to their previous employment,[3] and, following this report, the use of the method gained impetus with the consequent collection of a rapidly accumulating literature.

Most of the observers thus far have been German, Austrian, or English, but the method is now being used to a limited extent in the United States and a constantly increasing number of reports may confidently be expected.

That remissions occur following the inoculation of paretics with malaria seems indisputable; that they must be carefully evaluated, studied, and interpreted is apparent for, as stated by Solomon and Viets[4] in reference to the evaluation of methods of treatment in cerebrospinal syphilis: "A period of

years is necessary before one can be relatively or absolutely sure that he is not misinterpreting a remission as an arrest."

The treatment of paresis by malarial inoculation is purely empiric and the results without adequate or satisfactory explanation.

It has been suggested that an antagonism may exist between the Plasmodium malariae and the Spirocheta pallida as a result or consequence of which the reaction to malarial infecion brings about a production of antibodies having some degree of effectiveness against the spirochetae, but this suggestion is obviously purely speculative and rests upon no demonstrable basis.

Another view is that, as all the body processes, including those concerned with antibody production, are increased in velocity in the presence of fever, so the syphilitic foci may more actively produce antibodies, but this, also, is purely hypothetical.

Still another hypothesis is that, as malarial parasites are prone to seek lodgment in the minute capillaries of the brain, these vessels are hence apt to become more permeable and so permit protective substances to enter the brain more easily from the blood.[5]

Probably the majority of observers, however, attribute the effects entirely to the high fever induced. The modus operandi, however, remains unexplained for while Weichbrodt and Jahnel[6] found that, in rabbits, a complete disappearance of the spirochetae from scrotal chancres could be brought about by exposure to temperatures of 42° to 44° C. (107.6° to 110° F.), repeated not less than three times, Bunker and Kirby[7] state that the thermal death point of the Spirocheta pallida, "at least, in general paralysis," is 56° C. continued for ten minutes, although the organism fails to grow in temperatures above 40° to 41° C.

The observation of Hines is of some interest in this connection:

Nine days after his arrival from Santo Domingo, a man of thirty-five was admitted to the hospital with typical symptoms of severe malarial infection, the parasites of the aestivo-autumnal variety being found in the blood in large numbers. The cerebrospinal fluid was bloody and gave a strongly positive complement-fixation reaction in amounts as small as 0.1 c.c. Death occurred twelve days after admission. Microscopically, the capillaries of the brain were shown to be plugged with large numbers of parasites which were also found in the adventitial spaces. There were, in addition, definite histologic evidences of syphilis, the lesions, however, showing no signs of alteration as a result of the malarial infection.[8]

It is to be borne in mind, however, that improvement after malarial inoculation takes place slowly and it is possible that this case was too acute to permit such changes to occur, if they do take place.

Despite the fact that the treatment is purely empirical, a sufficient number of cases have now been accumulated to warrant the assumption that the results apparently following its trial are something more than a coincidence; their permanence remains to be seen.

In view of these findings and of the fact that the method may come into more frequent use, some résumé of the proper technic and the contraindications to its use, seems of interest.

The earlier the treatment is begun the better seem the results, but it must be

strongly emphasized that the use of the method is only justified in the hands of skilled and competent workers and where excellent hospital facilities are at hand.

As the main object in view is the induction of repeated attacks of hyperpyrexia, not all cases of paresis are suitable for treatment by malarial inoculation.

Dattner and Kauders[9] consider the method applicable to any case in which there exists no acute or severe chronic disease other than the syphilitic involvement of the nervous system. Patients of low vitality are unsuitable as unable to withstand a sufficient number of paroxysms; severe renal or splenic diseases, or severe and uncompensated cardiac lesions or myocardial degenerations are contraindications. Slight and compensated cardiac lesions are not contraindications but cardiac remedies must be employed in addition to the malarial inoculation.

"Cured" pulmonary tuberculosis is not a contraindication. Obese individuals must be closely watched and this is true, indeed, of all cases because of the ensuing loss of weight and strength, and especially because of the constant danger of delirium. Vigilant and constant supervision, therefore, is essential.

The technic of the inoculation is important as related to the number of successful "takes."

It is necessary that the donor shall have had at least one typical attack. The usual amount of blood injected by foreign workers is 4 cm. by the so-called "combined" method and 2 cm. by the intravenous method.

The "combined" method of injection, "imitating the movements of the anopheles mosquito," comprises the intramuscular, subcutaneous, intracutaneous, and extracutaneous inoculation of the malarial blood.

This is accomplished by introducing the needle at first deeply into the muscles and withdrawing it slowly after each inoculation until the last 15 or 20 drops are injected *on* the skin and inoculated by scarification.

If this method fails, the injection may be made intravenously.

When malarial paroxysms develop, the patient is permitted to have from ten to twelve paroxysms before the course of the disease is controlled by quinine, though some American observers have permitted a greater number in patients in good physical condition.

Occasionally, spontaneous cessation of the paroxysms occur before this number has been reached and in such instances the use of injections of milk or antityphoid vaccine has been suggested to provoke them.

Occasionally, individuals are found who seem refractory to inoculation though, perhaps, this may be due to errors of technic.

It has been stated that, as the multiplication of the plasmodia is asexual in man, as opposed to the sexual cycle in the mosquito, transmission of the disease from inoculated individuals need not be feared and precautions to prevent it are not necessary.[10] Until this is definitely established as a fact, however, careful protection of the patients by screening is advisable.

There is still some disagreement, also, as to whether or not to rely upon the malarial inoculations alone or to reinforce them with the coincident use of specific remedies. This is properly the subject of further experimentation and both

BOOK REVIEWS

(Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.)

*The Medical Department in the World War**

THE empyema experience of the U. S. Army during the World War was unique. Knowledge of the proper treatment of this condition was distinctly promoted. Fortunately the advances made in this field have been perpetuated in the volume under consideration. Three hundred and ninety-four pages have been devoted to empyema. This includes its epidemiology, pathology, roentgenology, bacteriology and treatment, particularly surgical. The illustrations, many of which are in colors, are splendidly done. This contribution need not await the next war to be of value in surgical practice.

Perhaps the most spectacular surgical feats of the war were in maxillary facial reconstruction. The surgical aspects of this work are presented with profuse illustrations.

Sections three and four are devoted to ophthalmology in the U. S. and in the A. E. F. respectively. The opportunity for study of unusual eye conditions both traumatic and nontraumatic, was enormous. The reconstruction work is discussed in great detail. We find a large number of colored illustrations of ophthalmoscopic findings.

The section devoted to otolaryngology is the least comprehensive in the volume and is essentially a statistical compilation.

Clinical Biochemistry†

A LABORATORY manual designed primarily for the use of the students of Melbourne University. It covers the ground traversed by the majority of volumes designed for similar purposes. The studies of blood chemistry and of renal function recommended by the author are scarcely as comprehensive as those used in this country. He does not incorporate the Folin-Wu system. Blood sugar is determined by MacLean's method, a very satisfactory colorimetric method which is quite similar to that developed in this country by Haskins and Holbrook. In the blood urea estimation he also uses MacLean's method. Beyond this, studies of kidney function are limited to the MacLean urea concentration test, the estimation of urinary diastase, phenolsulphonephthalein test and the indigo carmine test. The author remarks that the need for a colorimeter in using the phenolsulphonephthalein

*The Medical Department in the World War, xi, Part II. Prepared under the direction of Major Gen. M. W. Ireland, M.D. Surgeon General of the Army. Pp. 827. Cloth. Gov. Printing Office, Washington, 1924.

†Clinical Biochemistry. By Ivan Maxwell, M.D., M.Sc. Cloth. Pp. 124. Price 12 shillings, sixpence. Melbourne. W. Ramsay, 233 Lonsdale St., 1925.

test is a disadvantage to the general practitioner. The ease and accuracy with which a test tube colorimeter may be prepared at a moments notice impels us to question this statement.

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*Diagnostische Technik**

A MOST comprehensive reference work on the technic of diagnostic examination in all of its phases. Edited by Dr. Julius Schwalbe with contributions on special subjects and special methods of examination, from thirty contributors of recognized repute.

The first portion deals with general methods such as history taking, inspection, palpation, percussion, auscultation and exploratory puncture. In the second portion we find a most comprehensive program for clinical anthropologic measurements and studies of general body structure. There follow sections on the technic of roentgen examination, pathologic, histologic examination and of bacteriologic and immunologic studies.

The second section and by far the largest deals with various special methods of examination with such headings as blood, hemopoietic system, chest, heart and circulation, digestive tract, metabolism, kidneys, sexual function, the nervous system, surgery, gynecology, obstetrics, eyes, ears, nose, etc., venereal diseases, general infections, chronic tuberculosis, toxicology and pediatric examination.

This should serve as a very useful reference work

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*Diagnostische Technik für die arztliche Praxis. Ein Handbuch für Artze und Studierende. Herausgegeben von Professor Dr. Julius Schwalbe Geh. San. Rat. Mit 380 Abbildungen. Papier. Pp. 851. Leipzig 1923. Verlag von Georg Thieme

The American Society of Clinical Pathologists

The Society maintains a Service Bureau for its members. Any clinical pathologist wishing to make a change should communicate with the Secretary.

Kindly report any change of address to the Secretary.

The next annual Convention of the American Society of Clinical Pathologists will be held in Dallas, Texas, April 15, 16, 17, 1926.

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CLINICAL AND EXPERIMENTAL

THE DIAGNOSTIC VALUE OF THE CEREBROSPINAL FLUID SUGAR CONTENT*

BY W. P. STOWE, M.D., ROCHESTER, N. Y.

WITHIN the last few years numerous papers have appeared dealing with the sugar values of the spinal fluid in disease. Inasmuch as there has been some variation in the conclusions reached as well as in the methods used, this paper is offered as an analysis of 122 fluids of our own series, and 434 other reported cases, all using the Folin-Wu technic, essentially as for blood sugar.

Our counts have all been made with a Fuchs-Rosenthal chamber after dilution of the fluid with one-tenth volume of 15 per cent acetic acid, mixed in a white blood cell counting pipette. Strictly normal fluids never run counts higher than 4 cells per cu. mm. by this method.

Globulin estimations were all made by adding one to two drops of spinal fluid to a watch glass full of saturated aqueous phenol solution, the clouding being read immediately and graded from negative to four-plus, the latter figure representing the equivalent of pure blood serum so used.

Sugar determinations were made according to the Folin-Wu supplemental method,[1] with precipitation by the tungstic acid reagents except in negative Pandy cases, where 0.2 c.c. of spinal fluid, measured in an accurate pipette, was used without protein precipitation.

DIAGNOSIS

In the reported cases the diagnoses are believed highly reliable. All tuberculous meningitis fluids are from cases proved by necropsy or by re-

*Read before the Fourth Annual Convention of the American Society of Clinical Pathologists in Philadelphia, May 20-23, 1925
From the Rochester General Hospital, Rochester, N. Y.

covery of the tubercle bacilli from the spinal fluid by the Roby sedimentation method,[2] which we highly recommend for its ease, simplicity, and reliability of results.

Purulent meningitides were all proved bacteriologically. All brain tumor fluids were from necropsied or operatively proved cases. Neurosyphilitic cases all had strongly positive Wassermann reactions. The other diagnoses were based either upon necropsy or the concurrent opinion of more than one consultant.

RESULTS

1. *Normals.*—Ten fluids were examined that could be considered normal. All had three cells or less and negative Pandy and Wassermann reactions. These were obtained from patients upon whom lumbar puncture was performed as a routine upon discharge from salvarsan treatment. The range of this group was from 60 to 90 mg. per 100 c.c., the median point being 83 mg. This figure is higher than most reported normals, perhaps due to the clinic hours being such that puncture was done about three hours after morning or evening mealtime.

TABLE I

VARIED NEUROLOGIC CONDITIONS

	DIAGNOSIS	CELL COUNT	PANDY	SUGAR MG. %
1	Neurotrophic foot	2	Neg.	114
2	Acute poliomyelitis	185	1+	111
3	Chorea	4	±	101
4	Glioma cerebri, necrosis	365	3+	93
5	Multiple sclerosis	3	1+	91
6	Amyotrophic lateral sclerosis	4	Neg.	91
7	Epilepsy	2	Neg.	90
8	Multiple sclerosis	1	1+	90
9	Acute poliomyelitis	33	2+	88
10	Spastic paraplegia	3	1+	87
11	Acute poliomyelitis	91	1+	80
12	Hysteria	2	Neg.	80
13	Brain tumor	230	3+	76
14	Pernicious anemia	1	Neg.	70
15	Multiple sclerosis	2	Neg.	70

TABLE II

PURULENT MENINGITIDES

NO.	DATE	CELLS	PANDY	SUGAR MG. %	TYPE
1		Cloudy	3+	25	Pneumococcus
2		3,000	3+	None	Pneumococcus
3		1,200	4+	None	Pneumococcus
4		20,000	4+	None	Streptococcus
5		Purulent	4+	20	B. Influenza
6		3,600	3+	None	B. Influenza
7		95	4+	20	Pneumococcus ventriculitis
8		65	4+	20	Late meningococcus
9	10/9	2,000	2+	None	Meningococcus meningitis; treated, with recovery
	10/11	7,000	3+	None	
	10/31	80	2+	57	
	11/12	9	±	64	
10	8/12	4,000	3+	None	Meningococci found
	8/19	300	3+	30	Meningococcus meningitis; treated, with recovery
	8/23	220	3+	30	
	8/27	25	2+	72	

2. *Nonneurologic conditions.*—This group comprised fifteen cases of uremias, hyperthyroidism, trichiniasis, active syphilis and the like, with cell counts of four or less and Pandy not over 1+. The range in this series was from 50 to 100 mg. with the median point at 80,—essentially the same as the normal group.

3. *Varied neurologic conditions.*—This group was composed of fifteen cases of nervous system disease in which there were too few examples of any one disease to classify it separately. The individual values will be shown in Table I. The group range was from 70 to 114 mg.; the median point was 90.

4. *Neurosyphilis.*—This group of fifteen cases of tabes, paresis, and cerebrospinal syphilis, all with strongly positive Wassermann reactions, showed cell counts ranging from 3 to 302 cells and Pandy from negative to 3+. As no relationship between either cell count or Pandy and sugar level could be found, detailed listing of the cases seems unnecessary. The group range was from 60 to 91; the median point 77 mg.

5. *Lethargic encephalitis.*—Of this interesting group of twenty cases with cell counts up to 191 and Pandy reactions up to 3+, only two cases fell above the range of our group of normals and varied nervous conditions. In these two cases the sugar values were 160 and 182 mg. respectively; but the latter was in a fluid obtained from a moribund patient who died within two hours and it probably reflects the preagonal rise in blood sugar that has been ob-

TABLE III

SPECIAL CASES

DATE	DIAGNOSIS	CELL COUNT	PANDY	SUGAR MG. %
	(1) Obstructive hydrocephalus. (severe)			
	Rt. lat. ventricle	60	4+	None
	Left lat. ventricle	45	4+	Trace
	Lumbar	445	Trace	60
	(2) Obstructive hydrocephalus.			
10/2	Ventricular	1	Neg.	133
10/2	Lumbar	2	Trace	100
10/4	Left ventricle	0	Neg.	125
10/4	Right ventricle	1	Neg.	114
	(3) Spinal sarcoma, lower dorsal level, pressure, myelitis.			
1/8	Lumbar	None	4+	60
1/4	Lumbar	14	4+	55
1/4	Cisterna magna	2	1+	55
	(4) Tetanus, intraspinal antitoxin, purulent aseptic reaction.			
8/18	(before antitoxin)	2	Neg.	77
8/19	(after antitoxin)	16,000 (pus)	4+	70
8/20		4,200	3+	63
8/22		40	1+	71
8/24		40	1+	120
	(5) Cerebral abcess, asceptic "sympathetic meningitis," sterile fluid.	1,600 (polys)	2+	70
	(6) Compound fracture of frontal bone, aseptic sympathetic meningitis, sterile fluid.	450 (polys)	1+	60

served in patients dying of other nondiabetic conditions. The group range was from 60 to 182; the median point, 81.

6. *Tuberculous meningitis.*—Twenty-one proved cases of this disease showed a range of from 10 to 50 mg. with the median point of the series at 30; figures approached by no other disease such as poliomyelitis, encephalitis, neurosyphilis or meningism in which the cerebrospinal fluids often show globulin and cytologic reactions like those in this disease.

7. *Purulent meningitis.*—Detailed report of this group is given in Table II. The range in all except recovered meningococcic cases was from 0 to 25, the majority showing only a faint unreadable trace.

In Table III are shown a group of cases of considerable interest in regard to the factors other than blood-sugar level, concerned with cerebrospinal fluid sugar. Cases 1, 2, and 3 all represent some obstruction to the flow of the cerebrospinal fluid. The absence of sugar in the ventricles in the first case, is probably due to residual infection. The fact that nearly normal sugar value was found in this case at the lumbar level and that the sugar values were essentially the same above and below the obstruction in Cases 2 and 3 indicates definitely that sugar enters the cerebrospinal fluid at all levels of the system.

Cases 4, 5, and 6 show fluids with varying degrees of purulent reaction without infection of the fluid itself and with normal sugar values, indicating

TABLE IV

	COOPE (quoted)	COOPE (own)	WILCOX LYTTLE	FOSTER	STOWE	TOTAL
			Normals			
Cases		3		22	10	35
Range		Av. 72		Av. 53	60-90	
Med. Pt.					83	
			Assorted Neurologic			
Cases	55	69	33		14	171
Range	25-110	43-111	40-99		70-114	25-114
Med. Pt.	73	72	59		90	71
			Neurosyphilis			
Cases		19			15	34
Range		44-102			60-91	44-102
Med. Pt.		70			77	73
			Nonneurologic			
Cases					14	14
Range					50-100	50-100
Med. Pt.					80	80
			Encephalitis Lethargica			
Cases	92	11	16	39	20	182
Range	30-110	54-94	51-121	50-113	60-182	30-182
Med. Pt.	79	75	80	74	81	77
			Tuberculous Meningitis			
Cases	27	12	19	11	21	32
Range	10-60	14-55	10-81	1-41	10-50	1-50
Med. Pt.	33	25	29	25	30	27
			Purulent Meningitis			
Cases				5	14	19
Range				0-	.0-30	0-30
Med. Pt.				70	.01-	.01-

that the mere presence of pus cells up to 16,000 per cu. mm. does not diminish the sugar in the cerebrospinal fluid.

In Table IV, I have brought together my own series and those reported by Coope,[3] by Wilcox and Lyttle[4] and by Harold Foster[5, 6] together with summation of the entire groups, in tabular form.

CONCLUSIONS

1. While few compilations of figures seem available on normal fluids, the general trend of recent literature is to raise the figure ascribed to sugar content considerably above that given by the earlier French workers and by Foster, and place it in the neighborhood of 75 mg. per 100 c.c.

2. When series of cases of neurosyphilis, lethargic encephalitis and other neurologic and nonneurologic conditions, other than the meningitides, are examined, their deviation from the normal range and median point is found to be so slight as to be without diagnostic significance.

3. Tuberculous meningitis gives values so definitely above the purulent meningitis cases and so definitely below all other conditions that an almost certain diagnosis can be quickly made in this disease.

4. The occasional purulent fluids obtained after intraspinous therapy, in brain tumor cases with necrosis or hemorrhage near the ventricles, in sympathetic aseptic meningitis, etc., can be rapidly delimited by their normal sugar content from infective meningitis in which the causative organism may not be promptly found. The presence of pus cells alone in the cerebrospinal fluid does not reduce its sugar content.

5. Two of our cases are interesting in that obstruction to the flow of cerebrospinal fluid at the base of the brain in the one and at the lower dorsal spinal level in the other did not produce any essential difference in the sugar content above and below the lesion. This would suggest that the diffusion of sugar into the cerebrospinal fluid occurs throughout the cerebrospinal axis rather uniformly and not through the choroid plexus alone.

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DISCUSSION

Dr. Ward Burdick.—We have found the test for the sugar content of the cerebrospinal fluid a very useful procedure in the Children's Hospital, of Denver. Our findings correspond quite consistently with those of Dr. Stowe. In encephalitis the sugar is often, but not always, increased; in syphilis it is usually normal (70 to 80 mg. per 100 c.c. of cerebrospinal fluid), while in acute meningitides (tuberculous, pneumococcal, influenzal, streptococcal, etc.) it is invariably markedly reduced and in rare instances entirely absent.

Dr. John A. Kolmer.—Has Dr. Stowe studied the blood sugar in relation to variations in the spinal fluid sugar?

Has Dr. Stowe studied the fate of the sugar in the spinal fluid in tuberculous and meningococcus meningitis? Mestrezat and others have expressed the opinion that the sugar

is removed by the leucocytes but my own experiments have not substantiated this view and I was interested to observe that in some of his cases with sterile fluids but containing large numbers of leucocytes, the sugar content was within normal limits indicating that the cells do not receive the sugar but that its reduction in acute bacterial meningitis may be due to its utilization by the organisms.

Dr. A. S. Giordano.—I have noted with interest the normal variations found by Dr. Stowe for they closely correspond with mine and these figures differ from those published by others. I have had seven cases of tuberculous meningitis and of these, four had no sugar, one had 45 mg. and the others varied from 5 to 15 mg. per 100 c.c. All of these cases were proved to be tuberculous meningitis either at autopsy or by demonstrations of the tuberculous bacillus in the spinal fluid by smear or pig inoculation. My findings in a few cases of so-called encephalitis have been very unreliable, because the values fell within my normal limits. I believe that in order to establish the real value of this diagnostic procedure no case report should be accepted unless the diagnosis has been substantiated by demonstration of the causing organisms or by postmortem. I have more than once found that the clinical diagnosis of encephalitis was erroneous at autopsy even when such a diagnosis was made by a recognized neurologist. In general, I feel confident that given a clear spinal fluid with a sugar content below 45 mg. per 100 c.c. and absence of any demonstrable organisms, in all probability this is diagnostic of tuberculous meningitis. I do not believe that at present the determination of glucose in spinal fluid can be given any other diagnostic significance.

Dr. Stowe (closing).—"I was very much interested in the discussion, especially that of Dr. Giordano, whose results were similar to ours. We found no tuberculous meningitides with no sugar at all. In encephalitis lethargica I feel sure the range is not higher than that in normal fluids, and that the spinal fluid sugar has little diagnostic value in this condition. We have had few blood sugars to correlate with our spinal fluids. One patient with diabetic coma had 600 mg. of blood sugar and 500 mg. of spinal fluid sugar at the same time. As to the cause of the diminished sugar in the meningitides I have little to offer. I had thought that it might be due to the action of the cells present but this would seem disproved by the brain tumor and antitetanic serum reaction cases that had very high cell counts and normal sugar values. Against the idea of the bacteria using it up, some meningococcus cases with few organisms reduce the sugar to zero while a trace has been present in some pneumococcus cases with innumerable organisms. Also tuberculous meningitis contains relatively few organisms but the sugar is greatly reduced. Perhaps the answer is in altered permeability of the cerebrospinal vascular apparatus.

HOW CAN WE BEST PROMOTE THE OBJECTS CONTAINED IN ARTICLE TWO OF OUR CONSTITUTION?*

By Herman Spitz, M.D., Nashville, Tenn.

ARTICLE two of our constitution states the objects of our society. Without these we would have no bona fide reason for our existence. I will read these objects so that they will be fresh in our minds:

The objects of this society shall be:

"(1) To promote the practice of scientific medicine by a wider application of clinical laboratory methods to the diagnosis of disease.

*Read before the Fourth Annual Convention of the American Society of Clinical Pathologists, in Philadelphia, May 20-23, 1925.

"(2) To stimulate original research in all branches of clinical laboratory work.

"(3) To establish from time to time uniform standards for the performance of various laboratory examinations.

"(4) To elevate the scientific and professional status of those specializing in this branch of medicine.

"(5) To encourage a closer cooperation between the practitioner and the clinical pathologist."

Our activé membership is limited to graduates of medicine from recognized medical schools, who have specialized in clinical pathology for at least three years and who devote the major portion of their time to clinical pathology. A question which naturally arises is this: As physicians, who specialize in the field of clinical pathology, are we equally interested in all of the objects as enumerated or are some of us interested in one of the objects more than in some of the other objects? Stated in another way—are you interested in promoting the closer cooperation between practitioner and clinical pathologist as much as or more than in the stimulation of original research; or is each of us interested in elevating the status of some questionable laboratory or individual as much as he is in establishing uniform standards in laboratory work? The answer depends upon the particular field of clinical pathology in which we are engaged.

THE CLINICAL PATHOLOGIST

The clinical pathologist is a peculiar combination of physician and laboratory worker. As a physician, he must be familiar with all of the medical sciences, he must be able to diagnose, to treat, and to prognose. He must be willing to share the responsibility of a given case with the physician who calls on him for help. As a pathologist, he must be familiar with the processes of disease, and with the aid of laboratory methods he must be able to distinguish between the normal and abnormal, both in structure and function, and be able to tell why such abnormality in function or structure or both occurs.

Stedman defines a clinician as a practicing physician as distinguished from a pathologist or laboratory worker. The same authority defines a pathologist as one versed in the special study of morbid changes associated with disease. I do not find a definition of the compound word clinical pathologist, other than the one given by the Advisory Advertising Committee of the American Medical Association, reported in the *Journal of the American Medical Association,* December 2, 1922. "A Clinical Pathologist is a manipulator of fixtures and inanimate substances." It would thus seem necessary that this society define the terms clinical pathology and clinical pathologist so that the world may learn that there is some difference between a clinical pathologist and a technician, whose most proficient accomplishment may be the performance of an albumin or sugar test on urine, or the developing of an x-ray plate.

A clinical pathologist must be a physician, primarily and basically. He must not only know medicine but he must also be familiar with the allied

sciences; namely, chemistry, physics, biology, botany, mathematics, et cetera, and upon this broad foundation he places his structure of pathologic knowledge. Not the mere adding of two liquids in a test tube but the ability to interpret in terms of body activity the result of such a simple procedure is the function of a clinical pathologist.

Let us then consider this definition:

Clinical pathology is the science of interpreting morbid processes as determined by means of various laboratory aids and correlating them with clinical symptoms.

In order to fulfill this requirement we see that a thorough clinical knowledge is of prime importance and he who attempts clinical pathology without clinical knowledge and experience is falling short of the possibilities within his field. May I refer to a few examples to more forcibly present this thought?

A dermatologist saw a small growth in the flexor surface of a finger. It was removed and sent to a pathologist for diagnosis. The pathologist reported a giant cell sarcoma and without seeing the patient advised amputation. Fortunately other opinions were sought and the neoplasm proved to be a giant cell tumor of the tendon sheath, a growth well known for its benign character. The lack of clinical experience on the part of the pathologist was almost productive of serious results.

Another case from a different pathologist showing lack of clinical experience proved more serious. An enlarged submaxillary node was removed and the pathologist reported a lymphosarcoma. X-ray treatment was instituted and serious burns resulted. When the wound refused to heal, consultation was sought and the removed gland proved to be inflammatory from infection around an unerupted tooth.

The dermatologist calls the clinical pathologist into consultation in many confusing cases. A particular case may present lesions suggestive of blastomycosis, lupus, epithelioma or gumma. The clinical pathologist suggests the proper tests and from this knowledge should direct the investigative work into the proper channels in order to make a diagnosis as early as possible and also to avoid unnecessary work, so that the patient may be saved needless expense.

The ophthalmologist calls us into consultation in obscure eye conditions. Has the patient an optic atrophy due to nephritis, lues, brain tumor or diabetes? The clinical pathologist again suggests the advisable tests.

The surgeons want the clinical pathologist present at the operation, so that he can see the tissue in situ as well as after it is removed. The clinical pathologist frequently decides for the surgeon how extensive his operative work should be. May I refer to another case? A man in the middle forties presented himself with an enlarged testicle, with a diagnosis by the surgeon of a carcinoma. It proved to be a gumma. Had the clinical pathologist been consulted prior to the operation, instead of after it, the patient would have been saved this mutilating operation.

Another case: a man thirty-eight years of age was advised by two surgeons to have his leg amputated, one surgeon's diagnosis being tuberculosis of the knee, the other, carcinoma. In this case the clinical path-

ologist was called in and he cleared the case by proving it to be luetic; the ulcerations of four years standing responding promptly to proper treatment.

The internist depends upon our judgment in numerous cases. A case was diagnosed as pernicious anemia by a very competent internist, the blood work being done by his office laboratory assistant. Not satisfied, this patient sought other advice and it remained for the clinical pathologist to show that the pronounced anemia in this case was due to constant oozing from hemorrhoids that had escaped the internist's attention.

But why go on with this enumeration? I am sure that each of you who is in active clinical pathologic work can recall similar cases. My object in briefly referring to these few cases is to emphasize in as forceful a manner as lies in my power, that clinical acumen must be the basis of the clinical pathologist's work. This is the main contention in my paper and all I have to say in this paper is to support this contention. With no intention to ignore, slight, or belittle the tremendous amount of invaluable work being done by clinical pathologists in research work, most of which is being carried on in special institutions, I hold that the clinical pathologists in the forward ranks, those in active practice of clinical pathology, who daily see the general practitioners and their patients are the ones who sell clinical pathology to the profession at large.

DISTRIBUTION OF CLINICAL PATHOLOGISTS

In an effort to classify clinical pathologists according to the nature of their work, I was amazed at their wide distribution. They are found in all fields of medical activity. An attempt was made to classify them according to hospital connections but this led into so many subclassifications that I abandoned this method. Then too, the Council on Medical Education and Hospitals of the American Medical Association has recently (during the present year) undertaken a very broad survey of the laboratory situation throughout the country. This survey was undertaken "with the knowledge and advice of the officers of the American Society of Clinical Pathologists" and several other national organizations. (Letter dated April 13, 1925, signed by secretary of council.) It is hoped that the result of this survey will do much to clarify not only the laboratory situation but also establish the clinical pathologists in charge of these laboratories as something superior to a mere manipulator of test tubes and other inanimate substances.

Clinical pathologists are found in hospitals, in private laboratories, and a large number, like myself, dividing their time between the hospital laboratory and the private laboratory.

In hospitals, we find the clinical pathologists as a rule engaged in routine work. Some devote some part of their time to research. There are a large number who devote all their time to research and to teaching, these usually being connected with special hospitals, institutions, and schools.

Other clinical pathologists are found in federal, state, county, or city public health work, either as director or in charge of health laboratories. Most of these find little or no time for private laboratory work.

Still other clinical pathologists are found in industrial organizations; such as food distributing organizations, large milk plants, insurance companies, et cetera.

INTEREST AND DUTIES OF CLINICAL PATHOLOGISTS

Let us examine the interest and duties of the clinical pathologist in these various connections and see if they harmonize with our objects. Basically, as stated before, each of these clinical pathologists must be a physician. However, we find that those clinical pathologists who come most frequently and constantly in contact with the general practitioner and specialists and their patients are the ones who are most vitally concerned with the medical application of laboratory diagnosis, while on the other hand those clinical pathologists who are in administrative positions, who seldom if ever come in personal touch with the physician and his patient are concerned primarily with the technical features of laboratory work.

If this general survey is tenable, and I so hold, we can readily see that each of these groups of clinical pathologists cannot be equally interested in each or all of our objects.

The first object is: To promote the practice of scientific medicine by a wider application of clinical laboratory methods to the diagnosis of disease.

We should expect those clinical pathologists connected with hospitals and in private laboratories to be most vitally interested in this object. Those, who like the health board laboratory men and those in other administrative positions seldom come in contact with patients, would have only a purely academic and passing interest in this object.

The second object is: To stimulate original research in all branches of clinical laboratory work.

In this field the clinical pathologists connected with the so-called teaching hospitals, research hospitals, and special hospitals and schools would find their special field of interest.

The third object is: To establish from time to time uniform standards for the performance of various laboratory examinations.

It remains to be seen whether "uniform standards" is the ideal in our laboratory work. A standard once accepted would usually mean that it is the best. Accepting it as such would remove all incentive to find a better method and so we would defeat object two, just considered. Those most interested would be the clinical pathologists connected with the practical, everyday, active practice of clinical pathology; those who come in constant contact with clinical material, such as, clinical pathologists connected with hospitals, special institutions, and private and group laboratories. May I suggest "optional standards" as a better term meeting the situation?

Object four is: To elevate the scientific and professional status of those specializing in this branch of medicine.

In my opinion, we have in this object the possibilities of doing some really constructive work. To elevate our standards is a worthy and laudable undertaking. However, the mere stating of this object will not achieve results. We must outline the various ways by which we hope to attain this elevation in status and then adopt definite rules of procedure to enforce them.

The code of ethics of the American Medical Association is our code. A number of items in that code are inapplicable to our society. Many rules of conduct are missing therefrom that could and should be incorporated in our code.

Dr. Sondern and his Committee on Standardization have laid before us a plan for standardization as affects the clinical pathologist alone. We must not overlook the fact that we have to deal with hospitals, with other practitioners, with other pathologists, with our employers and employees, with patients and with the public. We should have definite rules governing our various relationships, and to that end I propose that this society adopt a new code of ethics that will include all the valuable features contained in the code of the American Medical Association and such other rules of ethical procedure as may be found advisable. The code should cover rules on advertising, on contracts with hospitals, our relationship with our technical help, and all of our contacts.

Object five is: To encourage a closer cooperation between the practitioner and the clinical pathologist.

Those clinical pathologists in private laboratories, group laboratories, private and general hospitals, special hospitals and some special institutions are the ones most interested in this object because they are the ones who come more frequently in contact with the general practitioner and his patients. This comprises the largest group in the practice of clinical pathology.

MOTIVE

What is our motive in adopting these objects? Is it altogether altruistic or is there an element of selfishness in our motive? We may well ask ourselves and then answer because sooner or later we will be asked this very question. If we consider the first and last objects we see a rather suggestive parallelism. The first object is "To promote a wider application of clinical laboratory methods" and the fifth object is to promote a "closer cooperation between clinical pathologist and practitioner." In the minds of some, this may mean but one thing and that is a bid for more work from the practitioner.

That thought may be justified and should be justified. How? By a closer personal application to our work; by giving more thought to clinical application than to theoretical interpretation; by being a consultant and not a mere "manipulator of constants and inanimate substances." By being a clinician first and then a pathologist—by living up to our name in the order in which we are named, clinical pathologist.

Note: It is to be hoped that the results of this survey will be published by the council before all interest in it is lost.

DISCUSSION

Dr. Frank M. Huntoon.—Again I want to come to the defense of common sense. We are here for three reasons: first, to make a decent living, second, to be a good clinical pathologist, third, to educate the medical profession to appreciate good clinical pathology and good clinical patholgists. I am glad to see a paper brought forth that infers that there is a selfish motive. We all have that motive. It is through the duration of that motive that real good clinical pathologists are made. In the education of the physician we have to carry it a considerable way to reach the mutual benefit that is possible.

THE PHARMACOLOGY OF BENZYL ALCOHOL AND ITS ESTERS*

V. A Pharmacologic Investigation of the Effect of Sodium Benzyl Succinate "Benzycin" and Sodium Dibenzyl Phosphate "Benzyphos" upon the Respiratory and Cardiovascular Systems

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In 1918 Macht[1] noted that benzyl benzoate and benzyl acetate produced a fall in arterial blood pressure in dogs and rabbits when injected subcutaneously, intramuscularly, intraperitoneally, or intravenously. Two years later he[2] reported favorable results with the use of benzyl benzoate clinically, in circulatory hypertension and angina pectoris. Recently Babcock[3] published a report on the use of benzyl benzoate in the treatment of over twenty cases of angina pectoris with, as he believes, beneficial results in six cases. Spach[4] likewise believes this drug to be beneficial in angina pectoris. In 1920 Mason and Pieck[5] published a report on a series of experiments performed by them, in which they found that benzyl benzoate injected intravenously in dogs was followed by a prompt, pronounced, and prolonged fall in blood pressure. This lowering of blood pressure, they believed, was due, not to its action upon the blood vessels, but to its action upon the heart.

Nielsen and Higgins,[6] in a series of papers reporting their results upon intravenous administration of benzyl compounds, support Macht's findings. In 1923 Macht[7] studied the pharmacologic and clinical effect of benzyl mandelate. He observed it to be very effective in lowering the blood pressure in both experimental and clinical cases. Recently the effect of benzyl benzoate and benzyl acetate upon blood pressure has been studied thoroughly by Gruber[8] upon dogs, cats and rabbits, and by Gruber and Shackelford[9] upon man. The former observer noted that benzyl benzoate, benzyl acetate, and benzyl alcohol did not lower blood pressure in dogs when given by stomach tube, even in amounts two hundred and ten times the customary dose for man. When given intravenously, these substances were noted to increase blood pressure in 8 per cent of the experiments and to decrease it in 90 per cent. The heart was the first portion of the cardiovascular system to be affected. The benzyl compounds in small doses decreased its volume, whereas large doses caused dilation. Like Musser,[10] and Allen and Sherrill,[11] Gruber and Shackelford[9] found benzyl benzoate to have no noticeable effect upon the blood pressure in their clinical cases of arterial hypertension.

Inasmuch as all the benzyl esters thus far studied, benzoate, acetate, mandelate, succinate, cinnamate, stearate, cyanide, salicylate, acetyl salicylate, phenolate, fumarate, para-amino-benzoate and monobenzyl barbiturate are

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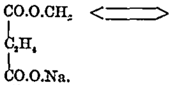
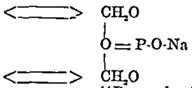
practically insoluble in water, and therefore, only slightly, if at all, soluble in the blood serum, it seems to us that intravenous administration of these drugs would be accompanied by undesirable side actions, the chief one among them being the formation of emboli. The quantity absorbed must also vary in proportion to the solubility of each drug so used. Recently there have been put on the market two double salts of the benzyl esters, which are soluble in water, "sodium benzyl succinate, benzycin, which contains about 39.5 per cent benzyl radical" and "sodium dibenzyl phosphate, benzyphos, which contains about 60.7 per cent benzyl radical."



"Benzycin"
Sodium benzyl succinate.



"Benzyphos"
Sodium dibenzyl phosphate.

Thus far I have been unable to find in the literature any report on the use of these preparations, nor have they, as far as I know, been accepted by the council on Pharmacy and Chemistry of the American Medical Association, for "New and Nonofficial Remedies."

According to the pamphlets sent to me by the manufacturers, these drugs have the same action as benzyl benzoate upon all smooth musculature and are indicated in all conditions in which benzyl benzoate would be used. They believe "benzycin" to be particularly valuable in "asthmatic conditions" and "benzyphos" to be of special use in relieving such conditions as "high blood pressure and uterine spasm."

This work was undertaken to determine the action of these drugs upon the respiratory and cardiovascular systems.

METHOD

The experimental animals used were dogs, cats, rabbits, terrapins, and frogs. The dogs were anesthetized with ether by inhalation or with paraldehyde (1.8 c.c. per kilo, per os.). The cats and rabbits were anesthetized with ether by inhalation or with urethane 2 gm. per kilo body weight by stomach. After anesthetization the animals were tracheotomized.

In the dogs, the injections were made directly into the exposed femoral vein. In the cats, the drugs were injected into the external jugular vein and also intraperitoneally, while in the rabbits they were injected into the lateral ear vein.

In all the experimental animals, except those in which perfusion was employed, the blood pressure was taken from a cannula inserted in the left carotid artery, and connected with a mercury manometer. A 2 per cent sodium citrate solution was used as an anticoagulant in those experiments in which the effects of the drugs were studied upon the respiratory systems, and in some of the experiments in which the drug was injected intraperitoneally. A sodium carbonate solution was used in almost all the remaining experiments. A chronograph marking 5, 10, 15, or 30 second intervals was

placed at the zero blood pressure level. Unless otherwise stated, all the writing points were placed one above the other.

The respirations in the dogs were recorded upon the kymograph surface by placing about the thorax a pneumograph connected with a Marey tambour. In the rabbits an S-shaped hook was fastened into the diaphragm through a median slit in the abdominal wall. By means of a ligature passing over pulleys, the S-shaped hook was connected to a writing lever so that the lever moved upward in expiration and downward in inspiration.

The organs of the abdomen were exposed through a mid-line incision. For plethysmographing a loop of intestine, or the kidney, metal oncometers were used connected either with piston recorders or with Gessell water tambours which marked the changes in volume upon the drum surface. The oncometers were applied with as little manipulation of the organs as possible and the abdomen then sewed up. A metal leg oncometer connected to a piston recorder was used in recording changes in the volume of the hind limb. In all instances, before the oncometer was applied the limb was disjointed at the ankle, so that the foot was not used in the plethysmographing oncometer. The limb and at least one other organ were plethysmographed simultaneously, and in many cases the volume changes of three organs were recorded at once, in addition to the recording of blood pressure.

After the establishment of artificial respiration, the changes in heart volume were recorded by placing a glass cardiometer about the heart, exposed by excising the ventral half of the thorax with the loss of as little blood as possible. Changes in blood pressure were recorded simultaneously. In a number of instances, records of both auricular and ventricular contractions were made simultaneously, with cardiographs[12] applied directly to the heart.

The hearts of dogs, cats, and rabbits were excised under ether anesthesia after exsanguination. These were perfused with Locke's solution, having a P_H of about 7.4, to which was added the defibrinated blood from the animal. In a number of dogs the kidney, loop of intestine, and hind limb were likewise perfused. The perfusion method was essentially the same as that used by Sherrington and Sowton.[13] The free end of the arterial cannula was connected to a short rubber tube leading from a reservoir containing the modified Locke's solution. This reservoir was kept in a water-bath at 38.5° C. and oxygenated by running oxygen through it. The perfusion pressure could be varied from 60 mm. of mercury to 100 mm. but was kept practically uniform throughout each experiment, by the rate of flow of oxygen into the perfusion reservoir. The excess oxygen was allowed to escape through a mercury valve which supported the desired pressure. During the escape of oxygen through the valve, the pressure would fall 4 mm. of mercury. This variation occurred about every five to ten seconds. Sodium benzyl succinate, or sodium dibenzyl phosphate dissolved in the perfusion mixture 0.1 gram per c.c., was injected into the perfusate near the arterial cannula against the perfusion fluid flow. Such injections had no effect upon the perfusion pressure as this was equalized by the escape of oxygen through the mercury valve.

The drops of perfusion fluid flowing from a glass cannula placed in the vein leading from the organ, were recorded on the kymograph by means of a receiving and recording tambour.

The hearts of rabbits and cats were perfused by inserting cannulas in the aorta near the organ; in the dogs, it was placed directly in the left coronary artery. The heart was immersed in the perfusion fluid in a glass cylinder. The excess fluid, as it escaped from the heart, was drained off and allowed to drop on a receiving tambour connected by means of rubber tubing to a Marey tambour, thus recording the rate of perfusion in drops. In a few cases, the heart was not surrounded by the fluid. The contraction of the heart was recorded on the kymograph by means of a light muscle lever connected by a ligature to the apex of the heart.

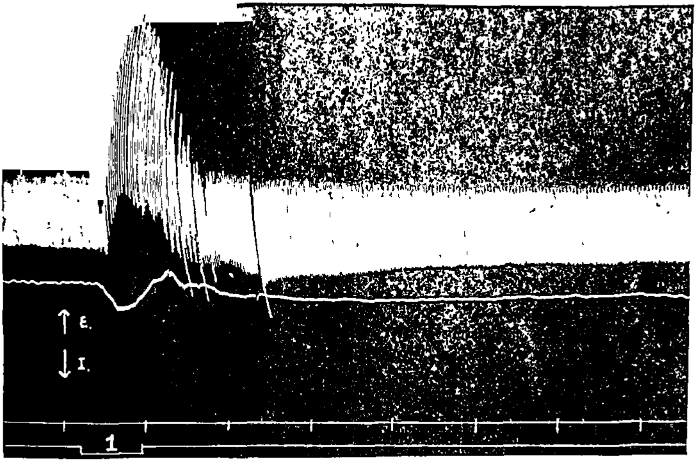


Fig. 1.—Rabbit, 1.7 kg., urethane anesthesia In this and the following two records the top record is that of respiration, and below it in order, the blood pressure, time interval in thirty seconds and zero blood pressure, and the time of the injection. In this and the following two records the upstroke ↑ *E*, in the respiratory record, indicates expiration; the downstroke ↓ *I*, inspiration. At 1, 0.8 gram sodium dibenzyl phosphate dissolved in 4 c.c. of water was injected intravenously. Three and a half minutes after the end of this record, the blood pressure was 80 mm. mercury.

The effects of these drugs upon the frog's heart and the terrapin's heart were studied also. The animals were always pithed and the heart exposed in the usual manner. The contractions were recorded by connecting the right auricle and the apex of the ventricle to light muscle levers. The heart was kept moist by a continuous drip of Ringer's solution. When the drug was tested, the Ringer's solution in which the drug was dissolved was substituted for the original Ringer's solution.

RESULTS

Solubility.—One gram of sodium succinate is soluble in 2 c.c. and an equal quantity of sodium dibenzyl phosphate is soluble in 6 c.c. distilled

water at room temperature 24.6° C. A solution of calcium chloride (0.024 grams per 100 c.c., added to the sodium dibenzyl phosphate solution invariably results in the formation of a fine white flocculent precipitate. This fact was first noted in an attempt to dissolve the drug in Ringer's solution. This precipitate is probably an insoluble salt of calcium phosphate. After dissolving the drug in distilled water, the addition of Ringer's solution similarly caused a precipitate to be formed. We must then assume that this reaction takes place in the presence of the calcium of the blood stream and, therefore, intravenous administration of this drug is a questionable procedure in clinical therapy.

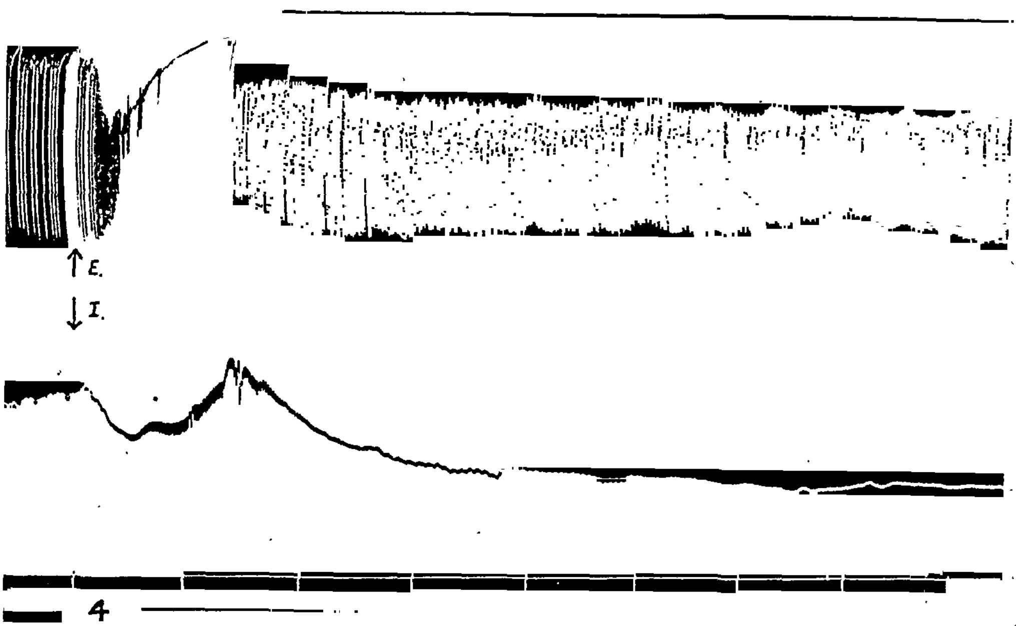


Fig. 2.—Rabbit, 1.8 kg., urethane anesthesia. Same as Fig. 1. At 4, 0.8 gram sodium dibenzyl phosphate dissolved in 4 c.c. of water was injected intravenously.

RESPIRATION

Like benzyl alcohol, acetate, and benzoate, sodium benzyl succinate and sodium dibenzyl phosphate when given intravenously cause in nearly all animals, an acceleration of the rate and a decrease in the depth of respiration. (Figs. 1, 2, and 3.) This is usually followed by a decrease in the rate with increased depth of respiration. Large doses produce paralysis of the respiratory center. (Figs. 2 and 3.) Whether the change in respiration is due to a direct stimulation of the respiratory center or a reflex stimulation due to the fall in blood pressure has not been determined. Mayer and Friedrich[14] found that a marked acceleration of respiration accompanies the fall in blood pressure, when amyl nitrite is given by inhalation. This they believed due to diminished blood flow through the respiratory center. Gesell, Capp, and Foote[15] noted an increase in respiratory rate when the blood pressure was lowered by hemorrhage, and concluded that the increase was due to the formation of acid within the center itself. It appears to me that there

may be a direct action upon the center with these benzyl compounds. In some cases the acceleration in respiration preceded the fall in blood pressure and in others, although the blood pressure did not fall appreciably and sometimes even rose, respiration was increased.

This acceleration in the respiratory rate is well shown in Figs. 1, 2, and 3, in which the upstroke of the lever indicates expiration and downstroke, inspiration. Figs. 1 and 3 were taken from the same animal, a rabbit weighing 1.7 kg. anesthetized with urethane. In Fig. 1, 0.8 gram sodium dibenzyl phosphate was administered intravenously at 1. There was an immediate increase in the rate and decrease in amplitude in respiration followed almost at once by slow, deep, forceful respirations which lasted about thirty seconds. Similar changes are observed in Fig. 3, in which 1 gram sodium dibenzyl

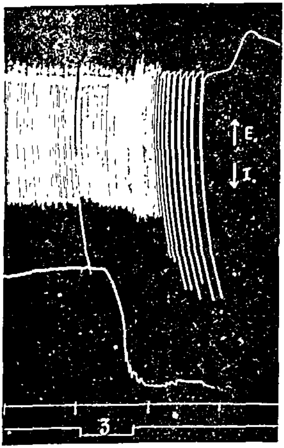


Fig. 3.—Rabbit, 1.7 kg., urethane anesthesia. Same animal as that used in Fig. 1. At 3, 1 gram sodium dibenzyl phosphate dissolved in distilled water was injected intravenously.

phosphate was injected. This injection caused permanent respiratory paralysis, probably of central origin. Upon stimulation of the phrenic nerve, after respiratory paralysis, the characteristic contraction of the diaphragm was still obtained.

A recovery from a temporary respiratory paralysis is observed in Fig. 2. This animal weighed 1 8 kg. Sodium benzyl succinate in one dose of 0.4 gram and one of 0.8 gram and sodium dibenzyl phosphate (0.4 gram) had been given intravenously before the injection seen in this figure. Following each of the preceding injections, there had been an acceleration in respiration, although the first injection caused a rise in blood pressure. In Fig. 2, 0.8 gram sodium dibenzyl phosphate was injected. There was total cessation of respiration for thirty seconds, accompanied by an asphyxial rise in arte-

rial blood pressure. A later injection of 1 gram of sodium dibenzyl phosphate caused permanent respiratory paralysis, the heart continuing to beat for some minutes.

BLOOD PRESSURE

Intravenous Administration.—The effects of sodium benzyl succinate and sodium dibenzyl phosphate upon blood pressure were not constant. In the main, there was a greater tendency toward a rise in blood pressure with smaller doses than with the larger ones. The effects that these drugs have upon arterial blood pressure may be seen in Figs. 1 to 5 and 8 to 10 inclusive and Tables I and II. In all the records except curves 5 and 9 fall in pressure is noted. With equal amounts by weight, this fall is more marked in every

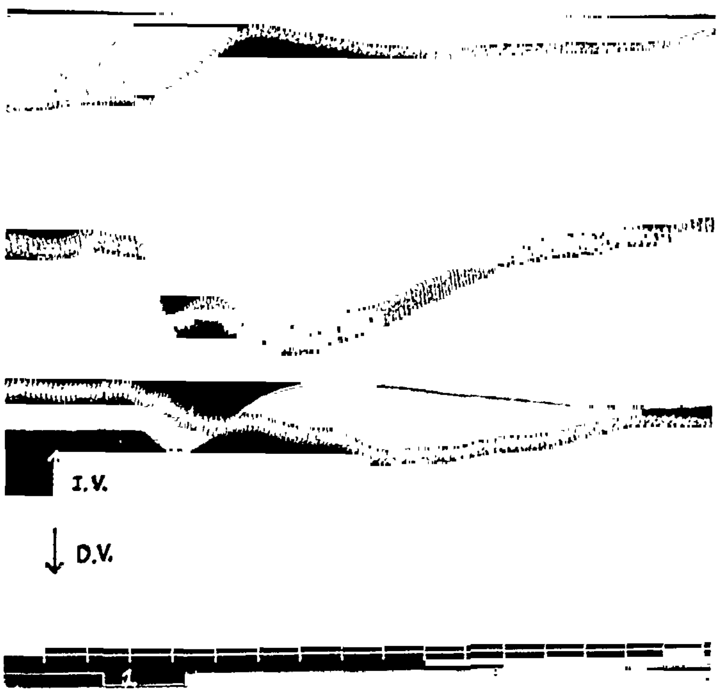


Fig. 4.—Dog, 17.7 kg., paraldehyde anesthesia. The top record is that of the plethysmographed intestine and below it in order, the blood pressure with mercury manometer, the volume of the limb, the kidney volume, the time in fifteen seconds and zero blood pressure, and time of the injection. The blood pressure writing point wrote 6 mm. in advance of the other three recorders. ↑ *I,V,* upstroke in the volume curves indicates increase in the volume and ↓ *D,V,* downstroke, decreased volume. At 1, 2 grams sodium dibenzyl phosphate dissolved in distilled water was injected intravenously.

case with sodium dibenzyl phosphate than with sodium benzyl succinate. In Table I, sodium benzyl succinate produced a fall in blood pressure in 20.3 per cent and a fall followed by a rise in 12.0 per cent of the injections. A rise was observed in 67.7 per cent of the cases. The fall in pressure varied from 3.2 to 35.5 per cent with an average of 14.7 per cent and the rise from 3.5 to 31.4 with an average of 13.0 per cent. Quite the opposite results were obtained with sodium dibenzyl phosphate. Fifty per cent of the injections produced a fall, 14.7 per cent a fall followed by a rise and only 35.3 per cent of the injections a rise in blood pressure. The percentile fall

in blood pressure varied from 8.0 to 66.6 with an average for the entire number of readings of 32.1 per cent. (Two readings are not included, as in these the blood pressure fell to zero and the animals died.) The percentile rise varied from 4.6 to 82.8 with an average rise of 17.3 per cent. See Table I. Somewhat similar results were obtained with rabbits and cats in which much larger doses were used. See Table II. In all the cases recorded in Tables I and II the increase in blood pressure following the injection of sodium benzyl

TABLE I

A SUMMARY OF THE RESULTS OBTAINED IN DOGS, UPON THE INTRAVENOUS INJECTION OF SODIUM BENZYL SUCCINATE AND SODIUM DIBENZYL PHOSPHATE. THE BLOOD PRESSURE READINGS ARE GIVEN IN MM. OF MERCURY

Grams sodium benzyl succinate per kilo. body weight	Blood pressure before injection	Blood pressure after injection	Fall in blood pressure in per cent	Rise in blood pressure in per cent	Grams sodium dibenzyl phosphate per kilo. body weight	Blood pressure before injection	Blood pressure after injection	Fall in blood pressure in per cent	Rise in blood pressure in per cent
0.02	92	86- 94	6.5		0.025	120	72	40.0	
0.055	92	88	4.3		0.04	143	124	13.3	
0.08	144	136-160	5.5		0.055	104	90	13.4	
0.11	96	66	3.2		0.055	106	90-110	15.1	
0.11	110	96	12.7		0.1	102	94-116	8.0	
0.13	102	84	17.6		0.11	170	94	44.7	
0.25	150	140	6.6		0.11	110	80	27.2	
0.25	150	140	6.6		0.11	102	72	29.4	
0.4	90	58	35.5		0.11	170	130-180	23.5	
0.4	88	62-100	29.5		0.11	160	104-176	35.0	
0.4	90	60- 99	33.3		0.13	152	104	31.5	
0.015	132	138		4.5	0.13	92	80	13.0	
0.03	62	76		22.6	0.15	160	0		
0.03	144	152		5.5	0.2	162	126	22.2	
0.03	60	78		30.0	0.2	120	40	66.6	
0.04	80	94		17.5	0.2	82	50	39.0	
0.04	130	142		9.2	0.2	96	58	39.6	
0.04	136	146		7.3	0.2	106	76-124	38.3	
0.05	78	95		21.8	0.22	100	45	55.0	
0.05	112	122		9.0	0.22	98	56	42.7	
0.055	86	94		9.3	0.25	154	84	45.4	
0.055	78	92		18.0	0.3	110	0		
0.055	70	92		31.4	0.015	128	134		4.6
0.06	110	126		14.5	0.02	122	136		11.4
0.077	135	150		11.1	0.03	150	163		8.6
0.1	94	106		12.7	0.03	100	110		10.0
0.11	90	100		11.1	0.03	90	100		11.1
0.12	133	142		6.7	0.03	100	104		4.0
0.2	88	102		12.5	0.04	132	147		11.3
0.2	152	164		7.9	0.035	98	110		12.2
0.22	188	204		8.4	0.06	126	144		14.3
0.22	168	174		3.5	0.08	80	98		22.5
0.23	142	168		18.3	0.14	58	106		82.8
0.25	146	156		6.8	0.25	140	160		14.3
Average fall in blood pressure in per cent			14.7					32.1	
Average rise in blood pressure in per cent				13.0					17.3

TABLE II

A SUMMARY OF THE RESULTS OBTAINED IN RABBITS AND CATS UPON THE INTRAVENOUS INJECTION OF SODIUM BENZYL SUCCINATE AND SODIUM DIBENZYL PHOSPHATE. THE BLOOD PRESSURE READINGS ARE GIVEN IN MM. OF MERCURY

GRAMS SODIUM BENZYL SUCCINATE PER KILO. BODY WEIGHT	BLOOD PRESSURE BEFORE INJECTION	BLOOD PRESSURE AFTER INJECTION	FALL IN BLOOD PRESSURE IN PER CENT	RISE IN BLOOD PRESSURE IN PER CENT	GRAMS SODIUM DIBENZYL PHOSPHATE PER KILO. BODY WEIGHT	BLOOD PRESSURE BEFORE INJECTION	BLOOD PRESSURE AFTER INJECTION	FALL IN BLOOD PRESSURE IN PER CENT	RISE IN BLOOD PRESSURE IN PER CENT
0.07	100	100			0.2	64	46	28.1	
0.25	96	(108-94)	2.6	12.5	0.2	68	37	45.6	
0.25	92	85	8.6		0.24	80	60	25.0	
0.25	86	96		11.6	0.25	80	84		5.0
0.25	100	96	4.0		0.25	77	84		9.1
0.5	92	36	61.0		0.37	68	20	70.7	
0.5	85	78-95	8.2	11.8	0.5	83	72	13.3	
0.6	82	94-38	53.7	14.7	0.5	72	(86-0)		19.5
0.075*	80	110		37.5	0.6	80	0		
0.075*	78	104		33.3	0.075*	92	70	23.9	
0.133*	90	116		28.8	0.133*	102	82-126	19.6	
0.333*	104	116-98-126	5.8	33.3	0.133*	104	78	25.0	
					0.33*	104	70	32.7	

*Cats under urethane.

succinate and sodium dibenzyl phosphate was a gradual one. In one curarized dog 0.14 gram per kilogram body weight of sodium dibenzyl phosphate, injected intravenously, increased the blood pressure in six minutes from 58 to 106 mm. of mercury, or an increase of 82.8 per cent. The heart rate increased simultaneously from 162 to 248 beats per minute.

Figs. 5 and 9 are presented to show the type of rise in blood pressure usually seen after the injection of these drugs. In Fig. 5, 4 grams sodium benzyl succinate, dissolved in distilled water, were injected intravenously in a dog weighing 17.7 kg. (0.22 gram per kilogram body weight). As a result of the injection the blood pressure was increased from 188 to 206 mm. of mercury, an increase of 8.4 per cent. In Fig. 4, 2 grams sodium dibenzyl phosphate was administered intravenously in the same animal before Fig. 5 was obtained. In this instance the blood pressure fell to 130 mm., but later rose to 180 mm. of mercury. In Fig. 9, a cat weighing 3 kg. was given 0.2 gram sodium benzyl succinate intravenously. The blood pressure increased from 78 to 99 mm. of mercury, or an increase of 27 per cent and the heart rate increased from 164 to 196 beats per minute, an increase of 32 beats. The blood pressure after nine minutes was still 90 mm. of mercury. Four-tenths of a gram of sodium benzyl succinate was later injected in this animal in a similar manner and the blood pressure rose this time from 88 to 116 mm. of mercury, an increase of 32 per cent. The heart rate was again accelerated, increasing from 184 to 204 beats, or 20 beats per minute.

A rise in blood pressure, following a fall, is observed in Figs. 1 and 2. In these, however, we attribute the rise in blood pressure not to the drug

injected, but to the asphyxia caused by the embarrassment of the respiratory mechanism as a result of the injection.

From the curves presented and from the collected data in Tables I and II, it will be seen that sodium dibenzyl phosphate, when given intravenously, appears to have a greater depressor action (depression of blood pressure) than does sodium benzyl succinate similarly administered. The dose appears to play an important part in the effect produced. Large doses cause a fall more often than do smaller ones and vice versa.

Intraperitoneal Administration.—The results obtained upon injecting sodium benzyl succinate and sodium dibenzyl phosphate intraperitoneally are interesting. Eleven cats under urethane anesthesia were employed. Twenty-four

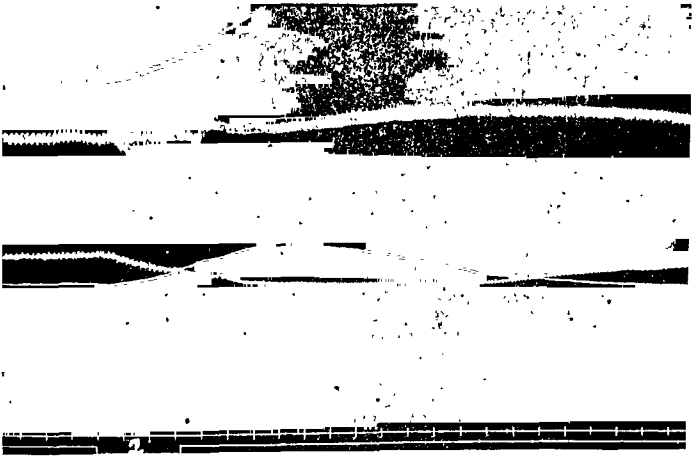


Fig. 5.—Continuation of Fig. 4. At 2, 4 grams sodium benzyl succinate dissolved in distilled water was injected intravenously.

injections were made, eighteen of which were with 0.5 to 1 gram of sodium benzyl succinate dissolved in 2.5 c.c. distilled water, and six with a similar quantity of sodium dibenzyl phosphate dissolved in 5 c.c. distilled water. In only a few cases 0.5 gram was used. Figs. 6 and 7 are typical of the results obtained from the sodium benzyl succinate injections. In all instances, within fifteen seconds after the injection, there was a sudden rise* in blood pressure varying from 10 to 75 per cent accompanied by dilation of the pupil. This rise was followed either by a gradual return to the control level as in Fig. 6 or after the return, by a second rise developing slowly and then gradually returning to the control level, as in Fig. 7. In a few instances the blood pressure

*Instead of the sudden rise in blood pressure in 8 rabbits similarly tested a sudden fall was observed in some cases as much as 40 mm. Hg.; otherwise, they responded in much the same way as cats.

creased, the limb volume simultaneously decreasing. The blood pressure increased from 188 to 206 mm. of mercury and the heart rate from 160 to 204 beats per minute. In this instance the dilation of the intestine was so great as to cause its recording lever to leave the drum's surface.

CARDIAC EFFECT

Intact Heart.—The results already presented show that sodium benzyl succinate and sodium dibenzyl phosphate like benzyl benzoate and benzyl acetate can increase the rate of heart beat. This stimulation appears only with small doses and more commonly after the administration of sodium benzyl succinate. When these drugs are given in large doses the heart is

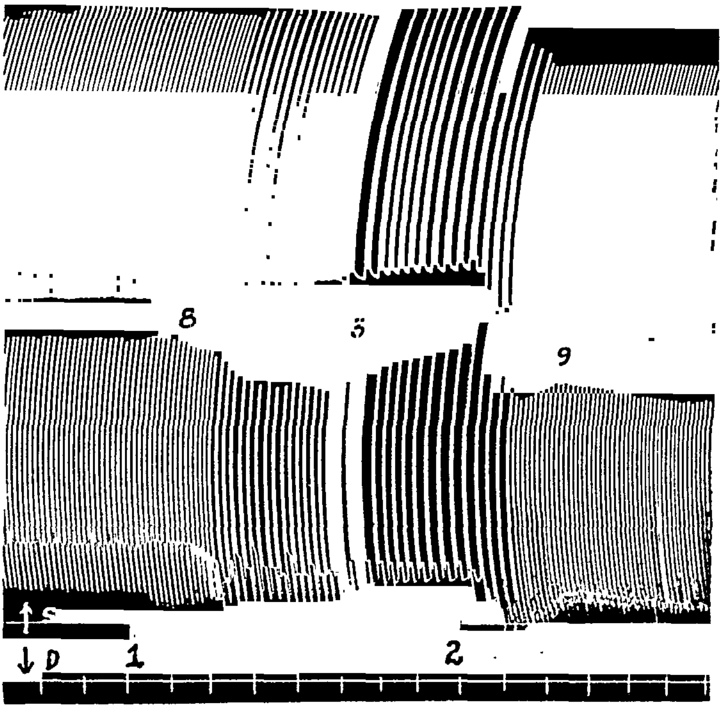


Fig. 11.—Terrapin, Chrysemys elegans. The top record is of contracting ventricle; below it in order are the records of the contracting right auricle and of the time that sodium benzyl succinate was dropped upon the heart, and the time interval in fifteen seconds. From points 1 to 2, 1 gram of sodium benzyl succinate dissolved in 2 c.c. of Ringer's solution was dropped on the heart. Upstroke ↑ *S*, indicates systole; the downstroke ↓ *D*, diastole. The heart rate is also indicated in fifteen second periods.

depressed. As further proof of this stimulatory action, experiments were performed upon intact and excised perfused hearts of dogs, cats, rabbits, and intact hearts of terrapins and frogs. Fig. 8 is a plethysmographic record of a dog's heart. The downstroke records systolic contraction, the upstroke, diastolic. At 1, 1.8 gram sodium benzyl succinate dissolved in 8 c.c. Ringer's solution was injected intravenously. The heart immediately dilated and the rate decreased 30 beats per minute. Within two minutes, however, the rate was normal and the volume of the heart was less than its original volume, i.e., the heart increased in tonus, and at the same time the excursion of the

lever showed the contractions to be more forceful. Such results were observed following most of the injection. Whenever the quantity injected was sufficient to produce a fall in blood pressure, the heart beat was slowed and the volume of the organ was increased. Smaller doses either had no effect or had a slight stimulatory action. Very large doses caused cessation of heart beat in which both auricles and ventricles stopped in diastole. Further confirmation of the accuracy of these findings was supplied by experiments in which cardiographs were applied to both auricles and ventricles. Figs. 9 and 10. In Fig. 9 (from a cat weighing 3 kg.), an increase in the force of muscular contraction, denoted by the increased excursions of levers attached

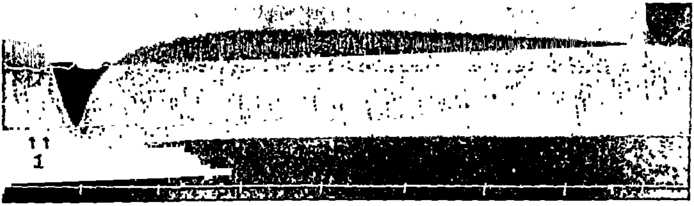


Fig. 12.—Isolated cat's heart perfused at 38.5° C. with Locke's solution, PH 7.4, to which was added about 15 c.c. of defibrinated autogenous blood per liter of fluid. The top record is the perfusion pressure in mm. of mercury; the middle record is that of the beating heart, and the bottom record that of the time interval in fifteen seconds and zero perfusion pressure. At 1, 0.1 gram sodium benzyl succinate dissolved in 2 c.c. of perfusion fluid was injected into the perfusion system near the aortic cannula.

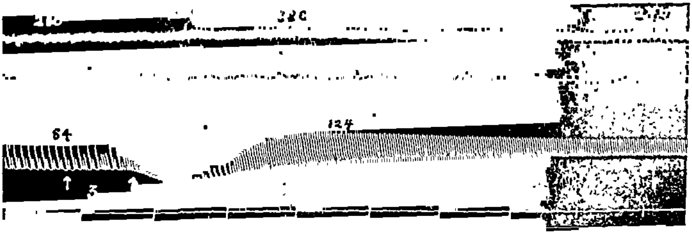


Fig. 13.—Same as Fig. 12. The top record is of the coronary flow in drops. At 3, 0.2 gram sodium benzyl succinate dissolved in 2 c.c. of blood Locke's solution was injected into the perfusate.

to both auricles and ventricles was noted after injecting 0.2 gm. sodium benzyl succinate. Here the effect appeared to be more marked in the auricles, but this was not always the case. In Fig. 10 is shown cardiac paralysis. In this instance 3 gm. sodium dibenzyl phosphate was injected intravenously in a dog weighing 10 kg. The heart dilated and finally ceased to beat, during which time the blood pressure dropped to zero.

Terrapin Heart.—In order to determine more accurately whether these drugs act as cardiac stimulants in small doses and cardiac depressants in large ones, they were tested upon intact terrapin and frog hearts by direct application and upon excised, perfused, mammalian hearts by injecting the

drug into the perfusate. It was found that dilute solutions of either drug when applied directly to the exposed terrapin heart had no effect. In a few cases the contractions appeared to become stronger but the results were so uncertain that no conclusions could be drawn. Strong solutions caused a decrease in the rate and in the extent of the excursion of contraction, which was always more marked in the auricles than in the ventricles. In Fig. 11, at 1, 2 c.c. of a 50 per cent solution of sodium benzyl succinate was dropped upon the heart, at 2 the benzyl solution was replaced by Ringer's solution. While exposed to the drug, the rate of contraction of the heart decreased from 8 to 3 beats per fifteen seconds. Upon replacing the benzyl derivative with Ringer's solution, the rate increased to 9 beats per fifteen seconds. A 16 per cent solution of sodium dibenzyl phosphate had a similar effect. In one case this strength of solution produced complete stoppage of the heart, from which it recovered after the drug was replaced by Ringer's solution.

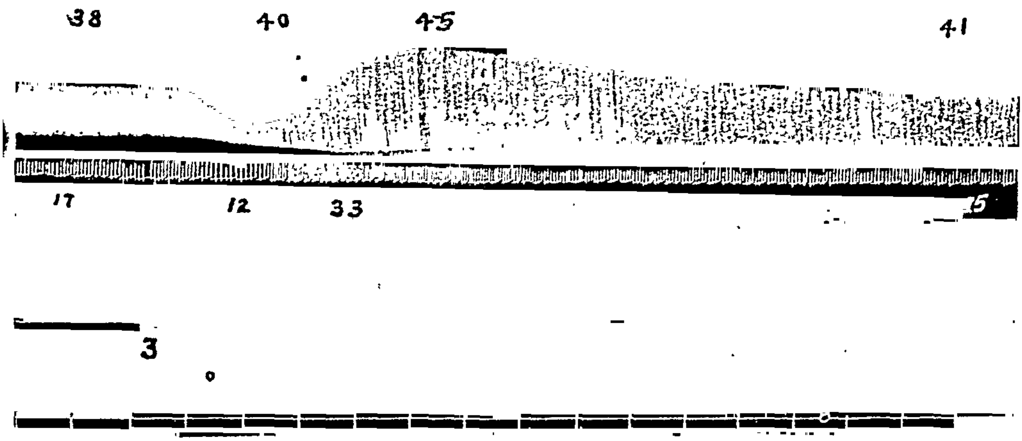


Fig. 14.—Excised rabbit's heart perfused with Locke's solution, P_H 7.4, was modified by the addition of defibrinated autogenous rabbit's blood, 10 c.c. per liter of fluid. Records from above downward are: cardiac contraction, coronary flow in drops, perfusion pressure in mm. of mercury, time of injection, and time interval in fifteen seconds and zero pressure for perfusion. At 3, 0.1 gram sodium benzyl succinate dissolved in 1 c.c. of modified Locke's solution was injected into the perfusate.

The results upon the frog's heart were similar to those upon the terrapin's except in one respect. In the frog acceleration of heart beat is occasionally seen. This was always followed by a decrease in heart rate.

Excised Mammalian Heart.—Figs. 12, 13, 14, and 15 show the cardiac stimulating and depressing actions of these drugs upon the mammalian heart. In Fig. 12, a cat's heart was perfused, at 80 mm. mercury pressure, with warm Locke's solution with a P_H 7.4 containing autogenous defibrinated blood. At 1, 0.2 gm. sodium benzyl succinate dissolved in 2 c.c. Locke's solution was injected into the perfusion fluid near the perfusion cannula. The heart immediately dilated and the force of contraction diminished. Later the force of contraction increased, the rate remaining the same. After the perfusion pressure had been increased to 92 mm. of mercury, another injection was made in the same heart. Fig. 13. The heart immediately dilated and became so slow it almost ceased to contract. This was followed by the disappearance

of the pulsus alternans seen before this injection, and the rate and force of contraction were increased. The heart rate which was 84 beats per minute increased to 124 beats while the coronary flow simultaneously increased from 200 to 380 drops per minute. Since here the betterment in heart action corresponded with a marked dilation of the coronary arteries, the question arises: is the betterment in cardiac action due to bettered nutrition or have these drugs a direct stimulating action upon heart muscle? In Fig. 14, a rabbit's heart was perfused at a pressure of 88 mm. of mercury, with warm Locke's solution P_H 7.4 containing autogenous defibrinated blood. In this case a temporary constriction followed the injection. This constriction can also be accounted for by the dilation of the heart, thus retaining a greater portion of the blood within the heart muscle itself. Such constriction occurred in a number of hearts in which the solution appeared to be either fairly large in quantity or concentrated. A marked dilation followed. The rate of perfusion flow first decreased from 17 to 12 drops per fifteen seconds

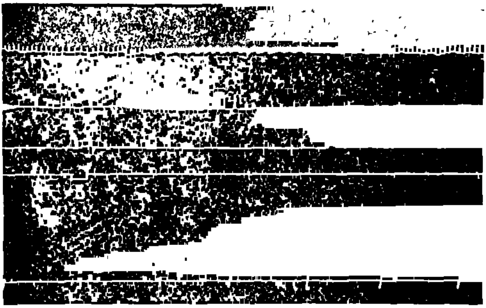


Fig. 15.—Excised rabbit's heart perfused with Locke's solution, P_H 7.4 was modified by the addition of 10 c c. of defibrinated autogenous blood to each liter of Locke's solution. The records from the top downward, show coronary flow in drops, cardiac contraction, perfusion pressure in mm. of mercury, time of injection of drug into the perfusate and time in fifteen seconds and zero perfusion pressure. x, vibration lever. At 3, 0.1 gram sodium benzyl succinate dissolved in 1 c.c. modified Locke's solution, was injected into the perfusate.

and then increased to 33 drops. The heart rate accelerated from 38 before the injection to 45 after. As in other records the decreased force of muscular contraction was accompanied by relaxation of muscle, i.e., dilatation. This increased coronary flow may be one of the factors concerned in the bettering of cardiac force but such results as those shown in Fig. 15, lead the author to believe that these drugs also have a stimulating action. In this experiment a rabbit's heart was perfused at 80 mm. of mercury pressure with warm Locke's solution P_H 7.4 to which was added defibrinated blood from the rabbit. At 3, 0.1 gm. sodium benzyl succinate dissolved in 1 c.c. Locke's solution containing blood, was injected into the perfusion system. Although the heart had stopped beating for some minutes after the injection it began to beat rapidly before any coronary dilation took place and ceased before the coronary flow had returned to its former rate. The rate of coronary flow in this case increased from 15 to 42 drops per fifteen seconds. In all cases so-

dium dibenzyl phosphate was found to produce greater dilation of the coronaries than did sodium benzyl succinate and it was also more toxic to cardiac muscle. Sometimes it caused cardiac acceleration, but in many instances, while the heart was still beating vigorously, it produced immediate and sometimes permanent cessation. Frequently the heart became arhythmic after its injection. In some instances there appeared to be established an A-V block and in others pulsus bigeminus, trigeminus, etc., and pulsus alternans.

In no case was the stimulating effect of these drugs equal to that produced by epinephrin in the same heart.

VASOMOTOR ACTION

Usually sodium benzyl succinate and sodium dibenzyl phosphate produced dilatation of the coronary arteries, when injected into the perfusate. This was demonstrated upon dogs, cats, and rabbits and occurred in both active and quiescent organs. A total of twenty injections of sodium benzyl succinate, varying in quantity from 0.1 to 0.5 gm., were made. An increase

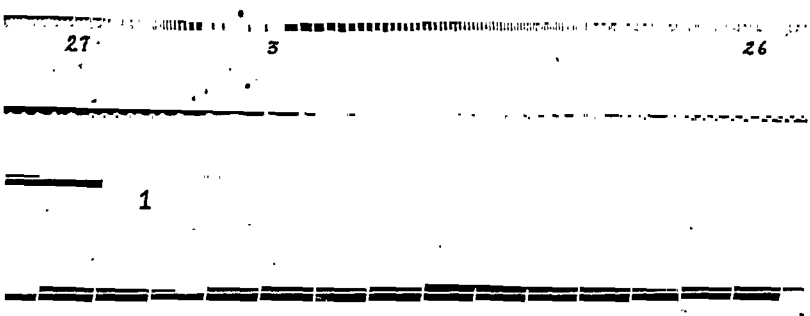


Fig. 16.—Cat, 3 kg., the animal was anesthetized with ether and exsanguinated. Right posterior limb was perfused with Locke's solution, PH 7.4, modified by the addition of defibrinated autogenous blood. The top record gives perfusion rate of flow in drops from the gracilis, adductor femoris, and semimembranosus muscles, and below it, the perfusion pressure in mm. of mercury. The bottom record shows time interval in fifteen seconds and zero pressure for the perfusion fluid, and above it the time of the injection. At 1, 0.025 gram sodium benzyl succinate dissolved in 0.25 c.c. modified by Locke's solution was injected into the perfusate.

in the rate of flow in drops from the heart was noted in the small doses and a decrease with large ones. Eighteen injections of sodium dibenzyl phosphate were made. In these a marked increase in rate of coronary flow was noted seventeen times. In one instance a large dose, 0.3 gm., produced a decrease in the coronary rate. Since this change occurred in both active and inactive hearts, it cannot, therefore, be attributed to increased activity of the organ. See Figs. 13, 14, and 15.

The effect that these drugs may have upon the rate of perfusion flow was also studied in the limb, intestine, and kidney. In the excised limb the gracilis, adductor femoris and semimembranosus muscles were perfused through the ramus muscularis branch of the femoralis artery[16] at a pressure varying from 60 to 80 mm. of mercury but constant for each experiment. Twenty-one injections were made and in these vasoconstriction, decreased rate of flow of fluid from the limb, occurred eighteen times. In three instances the rate was increased. Fig. 16 shows the usual result obtained from the limb.

At 1, 0.025 gm. sodium benzyl succinate was injected into the perfusate near the perfusion cannula. The rate of flow in drops decreased from 27 to 3 drops per fifteen seconds, but soon returned to its original rate. Loops of intestine were perfused, and in these, fourteen injections were made. The results here were somewhat more variable than those of the limb, but in the main, confirmed our plethysmographic findings. Increased rate of flow in drops was noted after the injections of these drugs in nine cases and decreased rate of flow in five. In the latter instances the quantity injected was usually large, 0.2 to 0.4 gm. The kidneys of three cats were perfused. In these, six readings were made. In all these the rate of flow in drops from the venous cannula was doubled after the injection of the drug. These results confirm our plethysmographic findings upon this organ.

The decreased rate in perfusion flow following the injection of concentrated solutions, we believe can be attributed mainly to the increased alkalinity of the injected fluid when either of these drugs is added to the perfusate and the action is that of increased P_H rather than that of a specific action of the drug.

DISCUSSION

Our results with the water soluble benzyl derivatives, sodium benzyl succinate and sodium dibenzyl phosphate, confirm our former views,[8, 9] that the benzyl esters are extremely unreliable for reducing blood pressure. Over 67 per cent of the injections of the former and 35 per cent of the injections of the latter resulted in increased blood pressure. Benzyl alcohol, benzoate, and acetate produced rises in blood pressure in 8 per cent of the injections.[8] We feel, as did Mason and Pieck,[5] that the point of action of the drug in those cases in which a fall in blood pressure results, is not the vessel wall only but also the cardiac muscle. Although these drugs can stimulate cardiac muscle, they probably are mainly cardiac depressants. If a sufficient dose is given to produce a fall in blood pressure, the first effect upon the cardiovascular system appears to be slowing the rate and dilatation of the heart with weakened contraction. The respiratory center is always first to be affected, in all injections. Usually there is acceleration in rate preceding the paralysis. It succumbs readily to comparatively small doses and is affected long before the heart is involved. The heart frequently beats some minutes after cessation of respiration. Occasionally an asphyxial rise in blood pressure is noted after cessation of respiration, showing that the vasomotor center has not been paralyzed simultaneously with the respiratory center.

SUMMARY

1. Sodium benzyl succinate is soluble in distilled water and Ringer's solution at room temperature (24.6° C.) in the proportion of 1 gram of the salt to 2 c.c. water or Ringer's solution.

2. Sodium dibenzyl phosphate is soluble in distilled water at room temperature (24.6° C.) in the proportion of 1 gram of the salt to 6 c.c. water. The presence of a calcium salt causes an insoluble flocculent precipitate to be formed. It is, therefore, insoluble in Ringer's solution and in the blood.

3. Both drugs appear to stimulate the respiratory center temporarily, causing an increase in rate with decreased depth followed by a slowed rate

and increased depth. Large doses cause paralysis of the respiratory center.

4. These drugs were injected 68 times in dogs, 8 times in cats, and 17 times in rabbits. The number of injections in dogs were equally divided between sodium benzyl succinate and sodium dibenzyl phosphate. Over 67 per cent of the injections of the former drug and 35 per cent of the latter produced rises in blood pressure. These rises averaged 13 and 17.3 per cent respectively. The remaining number of injections caused either a fall in blood pressure or a fall followed by a rise. Similar results were obtained in cats and rabbits.

5. Intraperitoneal injection of sodium benzyl succinate in cats causes a prompt increase in blood pressure, dilation of the pupil, and acceleration in respiration. In some cases the rise amounted to as much as 75 per cent. These changes we believe to be due to reflex stimulation of the entire sympathetic system, respiratory center, and possibly also the adrenal glands. The sudden increase is usually followed by a slow, progressive prolonged rise in blood pressure. In a few cases a fall was noted instead. Sodium dibenzyl phosphate also produced a sudden rise in pressure but this was followed in every case by a fall in pressure.

6. Intravenous injection of these drugs causes the volume of the intestine and kidney to increase but that of the limb to decrease.

7. Plethysmograph and myocardiograph records of the heart show these drugs, when injected intravenously in large doses, to cause dilation of the heart, slowed rate and finally cardiac paralysis. Small doses appear to increase the tonus and force and rate of contraction.

8. Perfusion experiments on the excised heart demonstrate these drugs to be cardiac stimulants in small doses and cardiac depressants in larger ones. With small quantities the increase in force and rate of contraction is always preceded by a decreased rate and force of contraction. In no case was the stimulation equal to that produced by epinephrin in the same heart. Large doses cause either temporary or permanent cessation of cardiac contraction. In some cases pulsus alternans, bigeminus, trigeminus and heart block appeared after the injection.

9. Local application of these drugs to the terrapin's heart and frog's heart causes a decrease in the rate and force of contraction of the heart. In a few frogs, an acceleration was noted.

10. Both drugs cause dilation of the coronary artery in small doses and constriction in larger ones. They also usually produce dilation of the intestinal and kidney vessels but constriction of the muscle vessels. The vasoconstriction we believe to be due mainly to the increased P_H of the fluid injected and not to a specific action of the drug.

11. Sodium dibenzyl phosphate was found to be more toxic and consequently more depressant to all tissues tested than was sodium benzyl succinate.

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GLYCOLYSIS AT VARYING BLOOD-SUGAR LEVELS*

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The phenomenon of the disappearance of the sugar from the blood in vitro has been studied repeatedly since Claude Bernard's time. Lepine[1] believed that the explanation of the nature of diabetes lay in the difference between normal and diabetic blood in this regard. He postulated the existence in normal blood of an enzyme causing the glycolysis; the absence or decrease of this enzyme in diabetic blood should account for the lessened rate or complete inhibition of the glycolysis. Many others have observed this lessened glycolysis in diabetic blood specimens. Denis and Giles,[2] for example, working with newer methods reported two years ago, that glycolysis is much more active in normal blood specimens than in the blood of persons suffering from fairly severe diabetes. Thalhimer and Perry[3] also published similar suggestive results. If this could be established as always true, it might be possible to develop a method of investigating and evaluating borderline or doubtful cases of diabetes mellitus. The validity of the proposition has already been rejected by Eadie, Macleod, and Noble,[4] and Cajori and Crouter,[5] and by Tolstoi.[20] The data herewith presented (Table I) indicate that while, in general, blood rich in sugar or diabetic blood seems to lose its sugar at a much less rate than do normal blood specimens, there is no such invariable characteristic behavior of normal and diabetic blood in this respect as would permit a differential diagnosis by means of studies of glycolysis rates.

There has also been a discordance of opinion as to the behavior of glycolysis at varying levels of blood sugar. Denis and Giles[2] considered that "the amount of glycolysis obtained, bears no relation to the concentration of the blood sugar." Cajori and Crouter[5] came to the conclusion that the rate of the glycolysis varies directly with the amount of sugar present. Macleod[6] found that glycolysis proceeds at the same rate in normal and in hyperglycemic blood. (Splanchnic stimulation and adrenalin.) Tsubura's[7] experiments, to which further reference will be made presently lead to the opposite conclusion. We have approached the problem from a somewhat different angle and our results will contribute additional evidence upon this point.

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TABLE I

GLYCOLYSIS IN BLOOD TAKEN FROM PATIENTS IN FASTING STATE

CASE NO.	BLOOD SUGAR MG. PER 100 C.C.			MG. LOST	PERCENTAGE LOST
	IMMEDIATE		AFTER 24 HR. REFRIGERATION		
2000	D*	83	20	63	76
2084	D	266	143	123	46
2104	D	250	210	40	16
2121	D	250	226	24	9
2127	D	138	111	27	19
1297	D	97	54	43	44
2139	D	182	166	16	9
2150	D	266	266	0	0
2159	D	200	200	0	0
2170	D	235	222	13	5
2183	D	200	190	10	5
2181	D	222	174	58	21
2195	D	286	235	51	18
2252	D	266	250	16	6
2411	D	210	185	25	20
2322	D	256	154	102	46
2354	D	166	65	101	60
2383	D	200	200	0	0
2371	D	133	10	123	90
2373	D	333	222	111	33
2386	D	285	210	75	27
2381	D	266	211	55	20
2265	D	166	129	37	23
2077	D	111	57	54	48
1174	D	200	153	47	23
2117	D	190	163	27	14
2214	N	89	46	43	48
1685	N	166	95	61	42
2262	N	160	105	55	34
2066	R	100	50	50	50
2092	R	111	66	45	40
2038	R	87	39	48	55
2178	R	105	80	25	24
2203	R	97	65	32	33
2221	R	100	66	34	34
1824	R	111	111	0	0
2261	R	100	23	77	77
1596	R	111	58	53	46
2365	R	85	10	75	90
2310	R	89	20	69	77
2025	R	117	46	71	61
2415	R	89	18	71	79
2342	N	166	83	83	50
2343	N	111	54	57	51
2402	N	117	46	71	61
97	N	125	89	34	29
1868	N	105	26	79	75
1909	N	105	50	55	52
2073	N	105	36	69	65
204	N	111	67	34	39
R. H. B.	N	133	91	42	31
2143	N	111	100	11	9
2167	N	91	83	8	9
2157	N	111	54	57	51
2164	N	133	80	50	40
2174	N	105	100	5	5
2147	N	133	105	28	21
2182	N	100	91	9	9
2158	N	111	100	11	9
2210	N	95	41	44	56
2193	N	91	53	38	43
27	N	105	77	28	27
2227	N	111	71	40	36
2259	N	111	81	30	27

*D=Diabetes mellitus, R=Renal glycosuria, N=Nondiabetic.

METHODS

A. The percentage loss of glucose after twenty-four hours was used as the *index* of glycolysis. We have not undertaken to determine the velocity of the process, but base our observations on changes in the relative rate of blood sugar loss.

B. Sugar determinations were made by the Folin-Wu method.

C. The blood specimens were oxalated to prevent coagulation, except where otherwise indicated. This procedure is not free from criticism, since it has been pointed out that there is reason to believe that oxalate exercises an inhibiting influence upon glycolysis.[5, 6] There is evidence in the work of Denis and Giles,[2] in that of Cajori and Crouter,[5] and in our own, that the inhibiting effect of oxalate is peculiarly intense in diabetic blood. When this peculiar influence of oxalate upon diabetic blood specimens is explained, a further step toward understanding glycolysis will be taken. We enter into no discussion upon this point at this time. We believe that the variation in the degree of inhibition cannot be attributed to the influence of oxalate when the relation of the latter in the different experiments is held constant. For further evidence upon this point, we have divided some blood specimens into several parts to which citrate and varying quantities of oxalate were respectively added. These mixtures were kept under identical conditions and observations were made as to the blood sugar loss. It would appear (Table II) that no important rôle should be attributed to the oxalate in the experiments to be presented later.

TABLE II

GLYCOLYSIS RATES OF DIABETIC BLOOD WITH CITRATE AND WITH VARYING AMOUNTS OF OXALATE (CASE 1692)

DATE	ANTICOAGULANT	BLOOD SUGAR IMMEDIATE	BLOOD SUGAR AFTER 24 HR.	MG. LOST	PERCENTAGE LOST
1925					
1/20	100 mg. citrate per 10 c.c. blood	228	228	0	0
1/20	20 mg. oxalate per 10 c.c. blood	228	228	0	0
1/20	40 mg. oxalate per 10 c.c. blood	228	228	0	0
1/27	100 mg. citrate per 10 c.c. blood	141	111	30	21
1/27	20 mg. oxalate per 10 c.c. blood	141	117	24	17
1/27	40 mg. oxalate per 10 c.c. blood	141	125	16	11
2/12	100 mg. citrate per 10 c.c. blood	121	111	10	8
2/12	20 mg. oxalate per 10 c.c. blood	121	118	3	2
2/12	40 mg. oxalate per 10 c.c. blood	121	121	0	0

D. The blood specimens were kept in a refrigerator at a temperature of from 9° to 11° Centigrade rather than in an incubator or at room temperature. This was done to minimize the possible influence that bacterial growth might have as a factor in causing glycolysis. While it has been repeatedly suggested that the disappearance of sugar from blood in vitro may be due to, or be materially influenced by bacterial contamination, investigators who have been careful to control their work bacteriologically have failed to demonstrate that the variations in glycolysis found by them could be ascribed to bacterial action.[5, 6, 7, 8, 20] These variations occurred when controls indicated perfect asepsis. Aseptic precautions were therefore considered superfluous. Attention is further directed to the fact (to be developed later) that

glycolysis was, in general, less in the blood specimens richer in sugar; whereas, if bacteria were responsible for the disappearance of the sugar, we would find their growth to be more luxuriant and their influence greater and, hence, the glycolysis more intense in the blood richer in sugar.

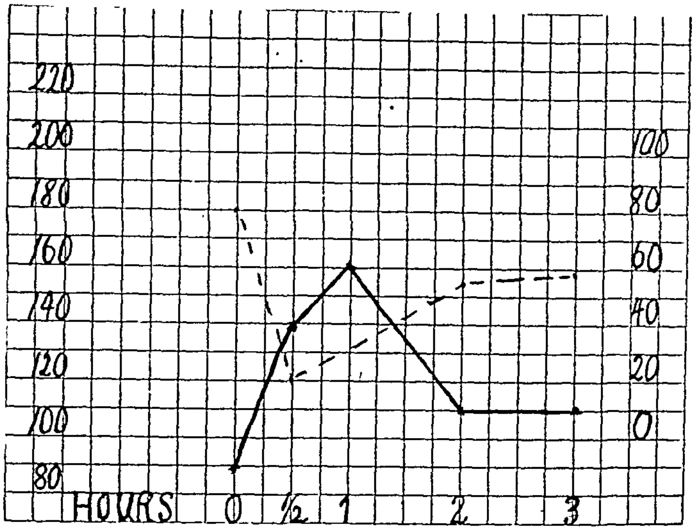

Chart 1.—Case No. 2415. Renal glycosuria. Glucose meal, 100 grams on fasting stomach. Typical relation between blood-sugar and glycolysis curves.

The *solid* line indicates blood sugar. Dotted line indicates percentage loss after standing 24 hours in refrigerator. *Figures at right indicate percentage of blood-sugar loss.* Figures at left indicate blood-sugar levels.

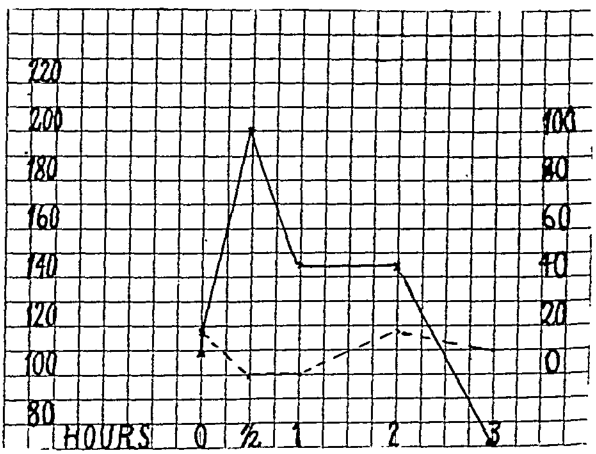

Chart 2.—Case No. 2359. Chronic nephritis. Arteriosclerosis. Hypotension. Glucose meal, 100 grams on fasting stomach. Marked rise in blood sugar and characteristic subsequent physiologic hypoglycemia. Characteristic glycolysis inhibition.

The solid line indicates blood sugar. Dotted line indicates percentage loss after standing 24 hours in refrigerator. Figures at left indicate blood-sugar levels. Figures at right indicate percentage of blood-sugar loss.

Most of our specimens were taken from patients in the fasting state (this was the case in all the specimens of Tables I and II). Occasionally, specimens were collected after a meal. The evidence suggested that alimentary hyperglycemia causes a slowing of the glycolysis rate in oxalated blood. The results are not invariable but, in general, where the blood sugar was not

TABLE III

GLYCOLYSIS IN BLOOD TAKEN BEFORE AND AFTER BREAKFAST (USUALLY COFFEE, MILK, SUGAR, PANCAKES AND SYRUP)

CASE NO.		BLOOD TAKEN BEFORE BREAKFAST BLOOD SUGAR MG. PER 100 C.C. IMMEDIATE	AFTER 24 HR. REFRIGERATION	MG. LOST	% LOST	TIME AFTER MEAL IN HOURS	BLOOD TAKEN AFTER BREAKFAST BLOOD SUGAR MG. PER 100 C.C. IMMEDIATE	AFTER 24 HR. REFRIGERATION	MG. LOST	% LOST
1596	R*	100	54	46	46	½	111	58	53	48
2292	R	100	44	56	56	½	118	71	47	39
2342	N	148	83	65	44	½	166	69	97	58
2371	D	133	20	113	85	½	250	200	50	20
2442	D	133	105	28	21	¾	333	250	83	25
2355	D	182	182	0	0	¾	222	222	0	0
2355	D	200	189	11	5 5	¾	235	230	5	2
2453	D	149	140	9	6	¾	190	183	7	4
E. P.	N	91	62	29	32	¾	105	80	25	24
R. T. L.	N	111	72	39	35	¾	91	77	14	15
I. I. L.	N	111	87	24	22	¾	117	83	34	29
2430	N	105	80	25	24	1¼	111	83	28	25
Miss D.	N	100	77	23	23	¾	105	88	17	16
Miss S.	N	105	83	22	21	¾	117	66	51	44
1596	R	100	54	46	46	½	111	58	53	48
2292	R	100	44	56	56	½	118	71	47	40
2343	N	105	66	39	37	½	125	103	20	16
2371	D	133	10	123	85	¾	250	200	50	20

*D=Diabetes mellitus, R=Renal glycosuria, N=Nondiabetic.

raised, the relative slowing of the glycolysis was not apparent. In Table III, the detailed results are given of blood specimens taken before and after a meal (usually pancakes, syrup, coffee, milk, and sugar). In striking contrast to the somewhat variable results quoted above are those obtained when hyperglycemia is produced by the ingestion of 100 grams of glucose, that is to say, during the course of the so-called blood-sugar curve or glucose tolerance test. In Table IV, there is seen in each case a slowing of the glycolysis rate with the rise of the blood-sugar level; as the hyperglycemia passes off, the glycolysis rate rises again. Charts 1, 2, 3, and 4 illustrate this point graphically. There is a divergence of the blood-sugar and glycolysis curves except in the case of Addison's disease (Chart 3), when no hyperglycemia occurred because of the increased tolerance for sugar. Chart 4 shows the low glycolysis rate usually found in diabetic blood, but even in this chart there is a divergence of the two curves. The most striking is Chart I where a scissor-effect is produced. This is the usual form of chart in normal individuals.

The slowing of the glycolysis with the rise of the blood sugar was put to the further experimental test by the intravenous infusion of glucose in two nondiabetic individuals. The results are similar to those where the glucose was administered by mouth. (See Table V.) In the third experiment exhibited in this table the peak of the glycemia was missed, because the blood specimen was taken too late and the inhibition of the glycolysis had passed off. It seems possible that if the blood had been taken earlier after the injection of the glucose solution, in both experiments on this patient, the results would have been more striking. Several intravenous infusions of glucose were given to a third patient, with chronic arthritis, and similar results were obtained. As the blood-sugar level rose, there was each time a fall

in the relative rate of the loss of blood sugar. Details are given in Table VI and one of the experiments is illustrated in Chart 5. In the first experiment there is a usual, but not striking, result. In the second experiment insulin

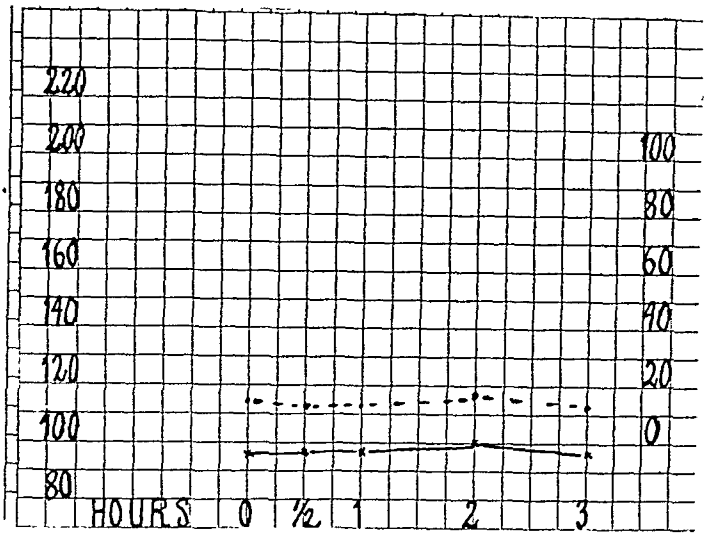

Chart 3.—Case No. 2471. Addison's disease. Increased tolerance for glucose. Glucose meal, 100 grams on fasting stomach. Flat blood-sugar curve. Flat glycolysis curve.

Solid line indicates blood sugar. Dotted line indicates percentage loss after standing 24 hours in refrigerator. Figures at left indicate blood-sugar levels. Figures at right indicate percentage of blood-sugar loss.

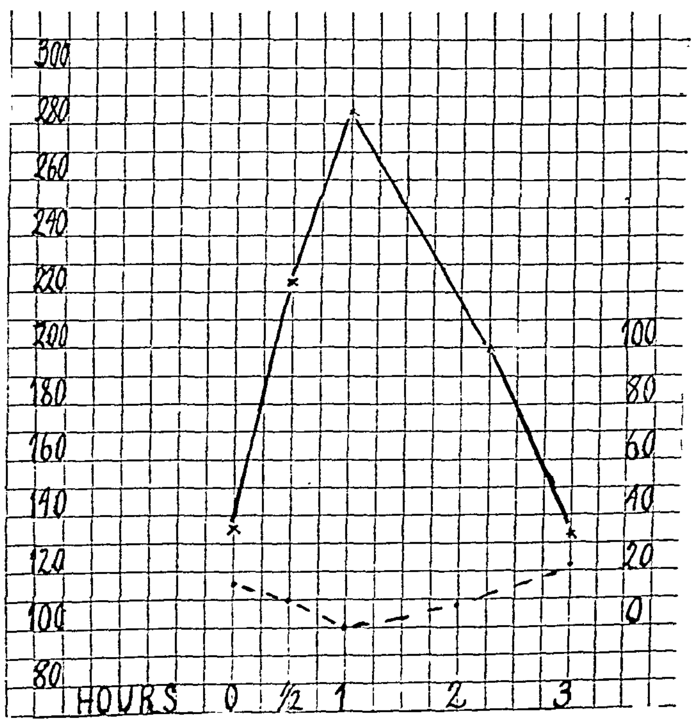

Chart 4.—Case No. 514. Mild diabetes mellitus. Glucose meal, 100 grams on fasting stomach. Low glycolysis rate throughout, the usual findings with diabetic bloods.

The solid line indicates blood sugar. Dotted line indicates percentage loss after standing 24 hours in refrigerator. Figures at left indicate blood-sugar levels. Figures at right indicate percentage of blood-sugar loss.

TABLE IV

GLYCOLYSIS IN BLOOD SPECIMENS TAKEN DURING BLOOD SUGAR CURVE AFTER MEAL OF 100 GRAMS OF GLUCOSE

CASE NO.		BLOOD TAKEN WITH RELATION TO MEAL	BLOOD SUGAR—MG. PER 100 C.C. IMMEDIATE	AFTER 24 HOUR REFRIGERATION	MG. LOST	PERCENTAGE LOST
2415	R*	Fasting	89	18	71	80
		½ hr. after	138	111	27	20
		1 hr. after	160	111	49	31
		2 hr. after	111	50	61	55
		3 hr. after	111	47	64	58
2310	R	Fasting	89	20	69	77
		½ hr. after	143	133	10	7
		1 hr. after	108	66	42	39
		2 hr. after	105	80	25	24
		3 hr. after	110	99	11	10
514	D	Fasting	133	111	22	16
		½ hr. after	222	200	22	10
		1 hr. after	285	285	0	0
		2 hr. after	200	182	18	9
		3 hr. after	133	105	28	21
2157	N	Fasting	80	53	27	34
		½ hr. after	114	50	64	56
		1 hr. after	80	77	3	4
		2 hr after	83	80	8	10
		3 hr. after	83	20	63	76
2471	A	Fasting	95	71	24	25
		½ hr. after	100	80	20	20
		1 hr. after	100	100	0	0
		2 hr. after	100	80	20	20
		3 hr. after	100	74	26	26
2471	A	Fasting	95	80	15	16
		½ hr. after	95	83	12	13
		1 hr. after	95	83	12	13
		2 hr after	100	83	17	17
		3 hr. after	95	83	12	13
2359	N	Fasting	111	95	16	14½
		½ hr. after	200	200	0	0
		1 hr. after	144	144	0	0
		2 hr. after	144	118	26	18
		3 hr. after	62	55	7	11

*D=Diabetes mellitus, R=Renal glycosuria, N=Nondiabetic

TABLE V

GLYCOLYSIS FOLLOWING INTRAVENOUS INFUSION IN NONDIABETIC PATIENTS (INFUSION = 20 C.C. OF 50% GLUCOSE SOL. = 10 GRAMS.)

DATE	PATIENT NO.	BLOOD TAKEN WITH RELATION TO INFUSION	BLOOD SUGAR IMMEDIATE	MG. PER 100 C.C. AFTER 24 HR. REFRIGERATION	MG. LOST	PERCENTAGE LOST
1924 10/23	T-6150	Before	80	66	14	17.5
		30 min. after	58	51	7	12
		75 min. after	100	66	34	34
10/21	T-4873	Before	83	67	16	18
		45 min. after	111	111	0	0
		60 min. after	80	65	15	19
11/6	T-4873	Before	95	20	75	79
		45 min. after	105	20	85	81
		120 min. after	100	40	60	60

was given hypodermically at the time of the glucose infusion, and there was not a subsequent hyperglycemia, but, in spite of this, the blood-sugar and glycolysis curves exhibit their usual divergence. In the subsequent experiments stronger glucose solutions were used; a hyperglycemia was produced and, with it, a more striking inhibition of the glycolysis. In the fifth experiment on this patient there was an initial postprandial hyperglycemia, 166 mg., which was raised to 222 mg. by the infusion, and the relative glycolysis rates were respectively 7 per cent and 5 per cent. A marked hypoglycemia followed (40 mg.) due in part to the insulin and in part, no doubt, to the physiologic effect of the glucose infusion. With this marked hypoglycemia there was a rise in the relative glycolysis rate to 32 per cent.

These results upon normal and diabetic individuals were obtained before the reports of the experiments of Tsubura,[7] published in June, 1924, had

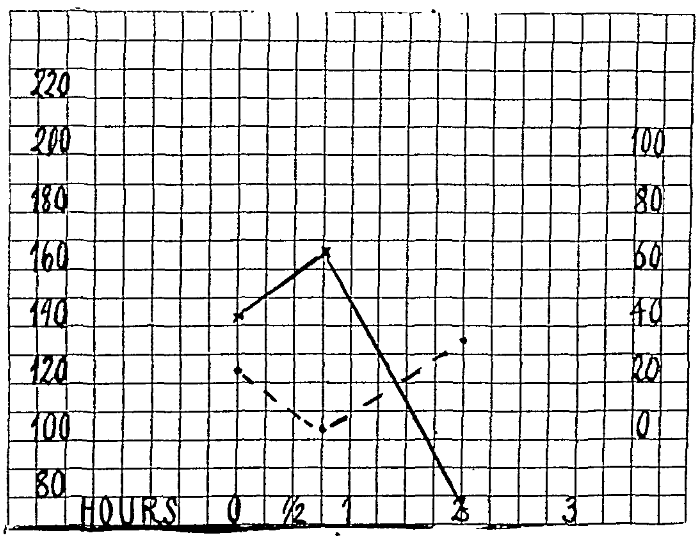

Chart 5.—Case No. 1876. Chronic arthritis. October 30, 1924. Intravenous infusion of 200 c.c. 20 per cent glucose. Insulin, 10 units hypodermically. Hyperglycemia and subsequent hypoglycemia. Typical relation between blood-sugar and glycolysis curves.

The solid line indicates blood sugar. Dotted line indicates percentage loss after standing 24 hours in refrigerator. Figures at left indicate blood-sugar levels. Figures at right indicate percentage of blood-sugar loss.

come to hand. His results upon the guinea pig blood are similar to those we have seen in human blood. He studied the glycolysis rate in defibrinated blood without the addition of any anticoagulant and compared the glycolysis of normal blood, (1) with blood fortified by the addition of glucose in vitro and, (2) with blood specimens of various hyperglycemic states. The latter were induced by adrenalin injections, by fourth ventricle punctures, by suffocation, by sodium chloride intravenous infusion, by caffeine, theobromin, and agurin injections, by restraint, and by hemorrhage. There was slight decrease in the glycolytic rate after the addition of glucose to the blood in vitro. When specimens of hyperglycemic blood were compared with normal blood fortified to the same sugar levels, it was found that there was evident glycolysis inhibition in the hyperglycemic states, except that following the injections of caffeine, theobromin, and agurin. The degree of in-

TABLE VI

GLYCOLYSIS IN BLOOD BEFORE AND AFTER GLUCOSE INFUSIONS WITH AND WITHOUT INSULIN IN A CASE OF CHRONIC ARTHRITIS

DATE 1924	GLUCOSE INFUSION	UNITS OF INSULIN		BLOOD SUGAR MG. PER 100 C.C.			
				BLOOD TAKEN BEFORE BREAKFAST	BLOOD TAKEN AFTER BREAKFAST	BLOOD TAKEN 45 MIN. AFTER GLUCOSE INFUSION	BLOOD TAKEN 2 HR. AFTER GLUCOSE INFUSION
I 10/27	200 c.c. of 10% Sol. (20 gm.)	0	Immediate	91		111	69
			After 24 hours	74		100	50
			Mg. lost	17		11	19
			Percentage lost	19		10	28
II 10/28	200 c.c. of 10% Sol. (20 gm.)	10	Immediate	83		83	57
			After 24 hours	62		77	50
			Mg. lost	21		6	7
			Percentage lost	35		7	12
III 10/29	200 c.c. of 20% Sol. (40 gm.)	10	Immediate		95	143	111
			After 24 hours		62	117	71
			Mg. lost		33	26	40
			Percentage lost		25	18	36
IV 10/30	200 c.c. of 20% Sol. (40 gm.)	10	Immediate		143	166	77
			After 24 hours		111	154	50
			Mg. lost		32	12	27
			Percentage lost		22	7	35
V 10/31	200 c.c. of 20% Sol (40 gm.)		Immediate		166	222	40
			After 24 hours		154	210	27
			Mg. lost		12	12	13
			Percentage lost		7	5	32

TABLE VII

GLYCOLYSIS RATES OF BLOOD OF DIABETIC PATIENTS WITH AND WITHOUT INSULIN

CASE NO.	BLOOD SUGAR MG. PER 100 C.C.				INSULIN
	IMMEDIATE	AFTER 24 HR. REFRIGERATION	MG. LOST	PERCENTAGE LOST	
2373	333	222	111	33	No
2373	222	87	135	60	No
1951	181	174	7	4	Yes
1749	91	74	17	19	No
1749	166	154	12	7	Yes
2386	285	210	75	26	No
2381	266	211	55	21	No
2381	235	200	35	15	Yes
1692	182	133	49	27	Yes
2402	160	154	6	4	Yes
2402	149	140	9	6	Yes
2355	182	182	0	0	Yes
2355	200	189	11	5.5	Yes

hibition was in direct ratio to the level of the hyperglycemia; as the latter rose, the glycolysis rate fell, and as the hyperglycemia passed off, the glycolysis rate rose again.

It would appear that glycolysis inhibition is not affected by insulin per se. This is in accord with the experiments of Eadie, Macleod, and Noble,[4] of Mauriac and Aubertin,[9] and others[10, 11] who have shown that no glycolytic action is to be attributed to insulin. In this connection, too, we exhibit, in Table VII, some observations upon glycolysis rates in diabetic patients, some

of whom were taking insulin and others of whom were not taking insulin. These also show that no apparent effect is to be attributed to the use of insulin.

The subject of glycolysis is still a fruitful field for investigation. Different blood specimens lose their sugar at different rates and the same blood loses its sugar at different rates under different circumstances. Whether this phenomenon is due to the action of an enzyme is still to be proved. It has been proved that the glycolytic power resides in the formed elements of the blood;[6, 12, 19] once the blood is laked with water, the glycolysis ceases. In this connection, we have speculated upon a possible relation between the physiologic leucocytosis occurring after meals and the glycolysis. It is evident at once, however, that the explanation cannot lie in this quarter, for a leucocytosis should bring about an increased glycolysis, not a decreased glycolysis as does occur after a meal. Tolstoi[20] and also Thalhimer and Perry[3] could find no correlation between leucocyte counts and glycolysis. Nor is it at all certain that the same glycolysis occurs in vivo as in vitro. Claude Bernard, Lepine,[1] Chauveau,[13] and other older authorities found less sugar in venous than in arterial blood and considered, therefore, that glycolysis occurred in the tissues. Later, Chauveau and Kaufman[13] and recently Mauriac and Aubertin[9] showed that the glycolytic power of the tissues is no less in depancreatized animals than in normal ones. The theory of Winter and Smith,[14] that there exist several forms of blood sugar, has not found substantiation at the hands of other investigators.[15,16,17,18] If their theory were true, the several forms of blood sugar might behave differently with respect to the process of glycolysis. The blood sugar in the general circulation after a meal or after a glucose infusion may be of a different nature from that which is given off slowly from the glycogen store of the liver. The *former sudden increment has not been stored and has not undergone* the changes incidental to such storing. All of this is pure speculation.

CONCLUSIONS

The present report indicates:

1. Glycolysis proceeds at a lessened rate when the level of blood sugar is raised, not only by glucose (or other food) ingestion, but also by intravenous glucose infusion.

2. The characteristic behavior of glycolysis in diabetic blood specimens may be related to the quantitative level of the blood sugar rather than to any essential quality of the blood.

3. Administration of insulin does not affect the characteristic behavior of glycolysis either in diabetic or in nondiabetic blood.

4. No important rôle is to be attributed to oxalate in evaluating the inhibition of glycolysis.

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THERAPEUTIC DYES*

I. Mercurochrome-220, Soluble

By R. W. French, Washington, D. C.

WITH the increasing demand for mercurochrome-220, soluble, the fact that its manufacture is not protected by patents, and that products of doubtful merit have been marketed under that name, need for means of analysis and standardization has become apparent. Early in 1924, on the assumption that a fixed chemical composition should result in a fixed dye and dye content, spectrophotometric methods of analysis were adopted. This method of study has been applied to mercurochrome-220, soluble.

From the examination of a number of samples submitted by Edwin C. White, the discoverer, it has been found that the genuine product as prepared under his supervision is extremely uniform and readily permits of the application of such methods of control. The accuracy of determination, both qualitatively and quantitatively, is believed to be quite high.

Chemically mercurochrome-220, soluble, is the disodium salt of dibromohydroxymercury-fluorescein or mercurated dibromfluorescein, the mercury probably entering into para position to the linking oxygen.[1] Thus, it is chemically somewhat similar to eosin.

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[1]White, E. C.: Jour. Am. Chem. Soc., 1920, xlii, No. 11.

Spectrophotometrically dibromfluorescein has a distinct maximum of absorption at 504.8 mμ according to Formanek[2] while eosin, according to the same author, has a maximum at 516 mμ. A tentative specific transmissive index determined for dibromfluorescein at 504.8 mμ is 1.54 while that of eosin at 516 mμ is 1.2155. Mercurochrome-220, soluble shows a distinct maximum of absorption at 512 mμ with a specific transmissive index from the mean of 13 samples of 0.804.

From this it will be noted that the addition of mercury to the dibromfluorescein has shifted the maximum of absorption toward the longer wave lengths of the spectrum 7.2 mμ and that there is relatively less color in the resultant chemical as evidenced by the lower value of the specific transmissive index.

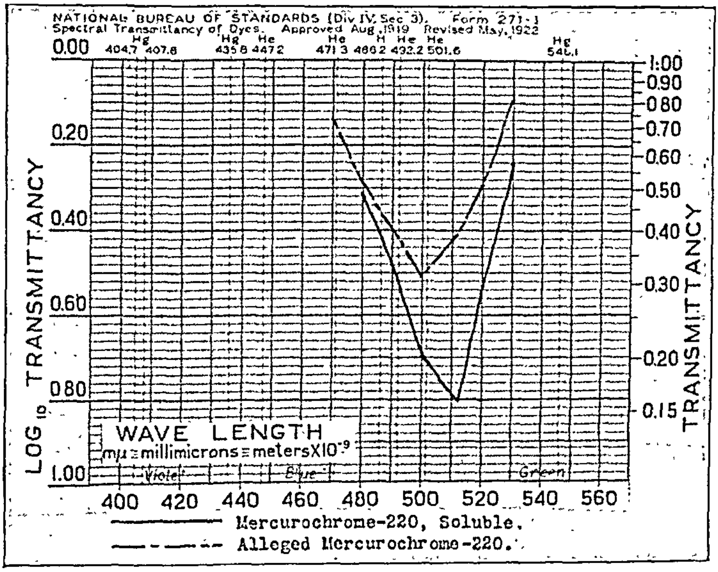

Fig. 1.

For the procurement of this drug the following specifications have been determined upon:

(a) Mercurochrome-220, soluble, shall be the disodium salt of dibromohydroxymercury-fluorescein.

(b) It shall be readily and completely soluble in water. Insoluble in alcohol (absolute).

(c) In general appearance the substance shall consist of greenish-appearing pseudocrystals and scales.

(d) On adding a few crystals to concentrated sulphuric acid a yellow solution is formed which on dilution with water gives a light orange precipitate. No change on adding to a 10 per cent aqueous solution of sodium hydroxide.

(e) Examined spectrophotometrically in a concentration of 10 parts of the dye to the million of phosphate buffer, P_H 7.0, a well-defined maximum

[2]Formanek, Untersuchung und Nachweis Organischer Farbstoffe auf Spektroskopischen Wege, Berlin, 1908.

shall occur at 512 mμ. The ratio between the transmissive indices at 500 mμ and 512 mμ shall be as 30 is to 34. For quantitative purposes such a solution shall show a transmissive index of 0.804 ± 0.050 at 512 mμ and of 0.700 ± 0.050 at 500 mμ when examined in a layer 1 cm. in thickness.

(f) When examined by methods common to the analysis of organic compounds, mercurochrome-220, soluble, shall show not less than 24 per cent or more than 26 per cent by weight of mercury on the dry basis.

Methods of inspection, tests, et cetera:

Mercurochrome-220, soluble, shall be tested according to the requirements of b, c, d, e, and f above. Values of the transmissive indices of a solution of mercurochrome-220, soluble, in phosphate buffer, P_H 7.0, 1.0 cg. of the dye to the liter of solvent when examined in a cell of 1 cm. thickness follows:

480mμ	0.322
490mμ	0.481
500mμ	0.700
510mμ	0.793
512mμ	0.804
520mμ	0.535
530mμ	0.240

These values should not vary more than a ±0.050 and there shall be no secondary maxima in the absorption curve. The maintenance of the ratio between the transmissive indices at 500 mμ and at 512 mμ as 30 is to 34 is important, as any increase in the value of the first member of this ratio indicates the presence of the color base, dibromfluorescein, which is undesirable.

The accompanying figure shows the curve of mercurochrome-220, soluble, plotted on the above values, as well as the curve of an alleged mercurochrome-220, soluble, which was on the market for a short time. It will be noted that this latter compound utterly failed to fill any of the above requirements and that spectrophotometrically it is in no sense the same substance. It showed 31 per cent mercury on analysis; and was only partially soluble in water.

Of the 13 samples submitted by White all met the above requirements easily and all had had clinical application.

POLYCHROME STAINS*

I. A Substitute for Giemsa's Stain

By R. W. French, Washington, D. C.

After considerable experimenting with, and study of numerous samples of azure I, and the resultant azure II and azure II-eosin on the market, the conclusion has been reached that the production of a consistent Giemsa stain by the older methods is out of the question and illogical. The azures never agree in composition, and it is not believed that any practical method for the alkaline oxidation of methylene blue will result in a uniform product. Oxidation plays too important a part in the reaction.

From the methods of alkaline reduction, attention was turned to the acid reduction methods which have been studied quite thoroughly by MacNeal.[1] Here definite products of lower methylation are obtainable from methylene blue and the processes are thoroughly reproducible. It also is practical to consistently manufacture methylene violet (Bernthsen) of a practical degree of purity.

Originally MacNeal's study showed that certain of the lower homologues of methylene blue with methylene violet are an essential in a satisfactory polychrome blood stain. This has been confirmed by the spectrophotometer, and it is noted that a stain in order to produce satisfactory results must have a certain definite proportion of the several blue elements. This is supplied by MacNeal in his tetrachrome blood stain by definite amounts of methylene blue, methylene azure A (MacNeal) and methylene violet.

Consideration of a Giemsa stain and the many difficulties experienced in its preparation in the customary manner from azure II and azure II-eosin in view of the marked variation of these components has lead to a solution of the problem in a manner similar to that used by MacNeal[2] in his blood stain. A substitute for Giemsa of the following composition is offered. This advantage is claimed: it has certain definite components and can be consistently prepared.

Eosin	1.250	gm.
Methylene Blue	1.250	gm.
Methylene Azure A (MacNeal)	0.750	gm.
Methylene *Violet (Bernthsen)*	0.250	gm.
Methyl alcohol, absolute	375.0	ml.
Glycerin (anhydrous)	125.0	ml.

The proportion of the components is the same as that used by MacNeal in the tetrachrome stain though the concentration is much greater to allow of

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the customary Giemsa technic. These proportions give the correct blue elements for a complete differential staining of the basic components.

While the stain can be prepared by the above formula, some difficulty may be experienced unless a special quality of glycerin is used—the usual samples of glycerin are not sufficiently dry. It is found that this quality in the glycerin is an important consideration. A freshly diluted stain will work better than one which has stood for some little time as the methylene violet precipitates out rapidly in aqueous solution, though it is an essential to a satisfactory stain.

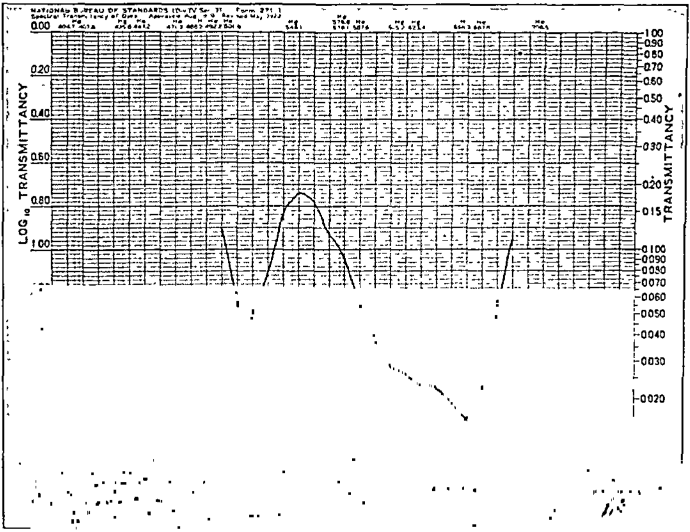

Fig. 1.

In use, a technic similar to that customarily employed with the original Giemsa is used; though controlled hydrogen-ion concentration is found to be advantageous for consistent results. In this latter method the above stain is used in the ratio of 3 ml. of stain solution to 50 ml. of a 50 per cent aqueous Sorensen phosphate buffer, P_H 7.5, and the slide is differentiated in a 50 per cent aqueous phosphate buffer of P_H 6.5.

Spectrophotometrically, as shown in the accompanying figure, this stain shows four distinct maxima of absorption: one at 516 mμ corresponding to eosin, one at 610 mμ due to the methylene violet, one at 630 mμ due to methylene azure and the greatest absorption at 660 mμ due to the methylene blue. Thus, the presence of the different elements is distinctly shown by this method of

analysis, and the relative amount of each may be ascertained. The study of different lots of stain compounded after the same formula shows a close agreement in the absorption curves and that the stain has a definite composition.

In another paper a discussion of the variations of Wright's stains, azures and Giemsa stains, on the market will be undertaken as revealed by the spectrophotometer.

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NOTES ON BASAL METABOLISM*

VIII. Tables of Values of the Du Bois Surface Area Formula†

By William H. Stoner, A.M., M.D., Philadelphia, Pa.

Du BOIS and Du Bois,[1] presenting their formula for human surface area, constructed a graph of values of the formula in order to obviate the necessity of the logarithmic calculation in routine determinations. This "height-weight" chart covered heights from 100 to 200 centimeters and weights from 20 to 110 kilograms, and has been copied[2-8] more or less accurately in varying sizes and covering varying ranges of height and weight. Boothby and Sandiford[9] constructed nomograms, which have been reproduced,[10, 11] for calculating the values of surface area according to the Du Bois formula.

Considerable difficulty is experienced in reading these graphs and nomograms accurately to three significant figures even though they be constructed to large scale. Sanborn[2] has tabulated to three significant figures the values of the surface area formula for heights 140 to 200 cm. by 5 cm. intervals, and for weights 30 to 150 kg. by unit kg. intervals.

Many cases encountered in these hospitals fall without these height and weight limits and must be calculated logarithmically from the formula. Moreover, the necessary interpolation of Sanborn's values is inconvenient. For these reasons the values of the Du Bois formula are here tabulated over a greater height and weight range and by unit cm. and kg. intervals. The values are recorded to four significant figures, although sufficient accuracy for clinical determinations is given by three figures, since an error of a unit in the third place introduces an error of less than 1 per cent in the basal metabolic rate.

The result of a basal metabolic rate determination, although expressed usually in one or in two digits, is in fact a three digit value. For example, a basal metabolic rate of +5 means that the rate of heat production of the subject is 105 per cent of the normal or expected rate. The 5, therefore, is in reality a third digit which, to be correct, must be derived from factors correct to four significant figures. For this reason the temperature, the pressure, the volume of oxygen consumed, the calorific value of one liter of oxygen, the normal or standard of comparison, as well as the surface area,—

*From the Biochemistry Laboratories and the Departments of Metabolic Diseases of the Philadelphia General Hospital and of the Graduate School of Medicine of the University of Pennsylvania.

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†Preceding Notes of this series appeared in the Boston Medical and Surgical Journal as follows:

I. Modified Clinical M[illegible] [illegible]xxix, 193.
II. A Simplified Data [illegible] 1923, clxxxix, 195.
III. Errors of Clinical [illegible]
IV. Selection of Norm [illegible]
V. Tables of Values [illegible] lx, 239.
VI. Complementary Tables of Values of Dreyer's Formulas, 1924, cxci, 1026.
VII. Actual versus Theoretic Weight in Dreyer's Formulas, 1924, cxci, 1030.

TABLE I

SURFACE AREA IN SQUARE METERS (DU BOIS FORMULA). 20 KG. TO 34 KG., 110 CM. TO 174 CM.

	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
110	0.775	0.791	0.807	0.823	0.838	0.852	0.867	0.881	0.894	0.908	0.921	0.934	0.947	0.959	0.971
111	0.780	0.796	0.812	0.828	0.843	0.858	0.872	0.886	0.900	0.914	0.927	0.940	0.953	0.965	0.978
112	0.785	0.802	0.818	0.833	0.848	0.863	0.878	0.892	0.906	0.920	0.933	0.946	0.959	0.972	0.984
113	0.790	0.807	0.823	0.839	0.854	0.869	0.884	0.898	0.912	0.926	0.939	0.952	0.965	0.978	0.991
114	0.795	0.812	0.828	0.844	0.860	0.875	0.889	0.904	0.918	0.932	0.945	0.959	0.972	0.984	0.997
115	0.800	0.817	0.833	0.849	0.865	0.880	0.895	0.909	0.923	0.937	0.951	0.965	0.978	0.991	1.003
116	0.806	0.823	0.839	0.855	0.871	0.886	0.901	0.915	0.929	0.943	0.957	0.971	0.984	0.997	1.010
117	0.811	0.828	0.844	0.860	0.876	0.891	0.906	0.921	0.935	0.949	0.963	0.977	0.990	1.003	1.016
118	0.816	0.833	0.849	0.865	0.881	0.897	0.912	0.927	0.941	0.955	0.969	0.983	0.996	1.009	1.022
119	0.821	0.838	0.854	0.871	0.887	0.902	0.917	0.932	0.947	0.961	0.975	0.989	1.002	1.015	1.028
120	0.826	0.843	0.860	0.876	0.892	0.908	0.923	0.938	0.952	0.967	0.981	0.995	1.008	1.022	1.035
121	0.831	0.848	0.865	0.881	0.898	0.913	0.928	0.943	0.958	0.973	0.987	1.001	1.014	1.028	1.041
122	0.836	0.853	0.870	0.887	0.903	0.919	0.934	0.949	0.964	0.978	0.993	1.007	1.020	1.034	1.047
123	0.841	0.858	0.875	0.892	0.908	0.924	0.939	0.955	0.970	0.984	0.999	1.013	1.027	1.040	1.053
124	0.845	0.863	0.880	0.897	0.914	0.930	0.945	0.960	0.975	0.990	1.004	1.019	1.033	1.046	1.059
125	0.850	0.868	0.886	0.903	0.919	0.935	0.951	0.966	0.981	0.996	1.010	1.025	1.039	1.052	1.066
126	0.855	0.873	0.891	0.908	0.924	0.940	0.956	0.972	0.987	1.002	1.016	1.031	1.045	1.059	1.072
127	0.860	0.878	0.896	0.913	0.930	0.946	0.962	0.977	0.992	1.007	1.022	1.037	1.051	1.065	1.078
128	0.865	0.883	0.901	0.918	0.935	0.951	0.967	0.983	0.998	1.013	1.028	1.043	1.057	1.071	1.084
129	0.870	0.888	0.906	0.923	0.940	0.957	0.973	0.989	1.004	1.019	1.034	1.048	1.063	1.077	1.090
130	0.875	0.893	0.911	0.928	0.945	0.962	0.978	0.994	1.009	1.024	1.039	1.054	1.069	1.083	1.096
131	0.880	0.898	0.916	0.934	0.951	0.968	0.984	0.999	1.015	1.030	1.045	1.060	1.075	1.089	1.103
132	0.885	0.903	0.921	0.939	0.956	0.973	0.989	1.005	1.020	1.036	1.051	1.066	1.080	1.095	1.109
133	0.889	0.908	0.926	0.944	0.961	0.978	0.994	1.010	1.026	1.042	1.057	1.072	1.086	1.101	1.115
134	0.894	0.913	0.931	0.949	0.966	0.983	0.999	1.016	1.032	1.047	1.062	1.077	1.092	1.107	1.121
135	0.899	0.918	0.936	0.954	0.972	0.989	1.005	1.021	1.037	1.053	1.068	1.083	1.098	1.113	1.127
136	0.904	0.923	0.941	0.959	0.977	0.994	1.010	1.027	1.043	1.059	1.074	1.089	1.104	1.119	1.133
137	0.909	0.928	0.946	0.964	0.982	0.999	1.016	1.032	1.048	1.064	1.080	1.095	1.110	1.125	1.139
138	0.914	0.933	0.951	0.969	0.987	1.004	1.021	1.037	1.054	1.070	1.085	1.101	1.116	1.131	1.145
139	0.919	0.938	0.956	0.975	0.993	1.010	1.027	1.043	1.060	1.076	1.091	1.107	1.122	1.136	1.151

140	0.923	0.942	0.961	0.980	0.998	1.015	1.032	1.049	1.065	1.081	1.097	1.112	1.127	1.142	1.157
141	0.928	0.947	0.966	0.985	1.003	1.020	1.037	1.054	1.071	1.087	1.103	1.118	1.133	1.148	1.163
142	0.933	0.952	0.971	0.990	1.008	1.026	1.043	1.060	1.076	1.092	1.108	1.124	1.139	1.154	1.169
143	0.937	0.957	0.976	0.995	1.013	1.031	1.048	1.065	1.081	1.098	1.114	1.130	1.145	1.160	1.175
144	0.942	0.962	0.981	1.000	1.018	1.036	1.053	1.070	1.087	1.103	1.119	1.135	1.151	1.166	1.181
145	0.947	0.967	0.986	1.005	1.023	1.041	1.059	1.076	1.092	1.109	1.125	1.141	1.157	1.172	1.187
146	0.951	0.971	0.991	1.010	1.029	1.047	1.064	1.081	1.098	1.115	1.131	1.147	1.163	1.178	1.193
147	0.956	0.976	0.996	1.015	1.034	1.052	1.069	1.086	1.103	1.120	1.136	1.152	1.168	1.184	1.199
148	0.961	0.981	1.001	1.020	1.039	1.057	1.074	1.092	1.109	1.126	1.142	1.158	1.174	1.190	1.205
149	0.966	0.986	1.006	1.025	1.044	1.062	1.080	1.097	1.114	1.131	1.148	1.164	1.179	1.195	1.210
150	0.971	0.991	1.011	1.030	1.049	1.057	1.085	1.102	1.119	1.136	1.153	1.169	1.185	1.201	1.216
151	0.975	0.996	1.016	1.035	1.054	1.073	1.090	1.108	1.125	1.142	1.159	1.175	1.191	1.207	1.222
152	0.980	1.001	1.021	1.040	1.059	1.078	1.096	1.114	1.131	1.148	1.165	1.181	1.197	1.213	1.228
153	0.985	1.005	1.025	1.045	1.064	1.083	1.101	1.119	1.136	1.153	1.170	1.187	1.203	1.218	1.234
154	0.989	1.010	1.030	1.050	1.069	1.088	1.106	1.124	1.141	1.158	1.175	1.192	1.208	1.224	1.240
155	0.994	1.014	1.035	1.055	1.074	1.093	1.111	1.129	1.147	1.164	1.181	1.198	1.214	1.230	1.246
156	0.999	1.019	1.040	1.060	1.079	1.098	1.116	1.134	1.152	1.169	1.186	1.203	1.220	1.236	1.251
157	1.003	1.024	1.045	1.065	1.084	1.103	1.121	1.139	1.157	1.175	1.192	1.209	1.225	1.241	1.257
158	1.008	1.029	1.050	1.070	1.089	1.108	1.127	1.145	1.163	1.181	1.198	1.215	1.231	1.247	1.263
159	1.013	1.034	1.054	1.074	1.094	1.113	1.132	1.150	1.168	1.186	1.203	1.220	1.237	1.253	1.269
160	1.017	1.038	1.059	1.079	1.099	1.118	1.137	1.156	1.174	1.192	1.209	1.226	1.243	1.259	1.275
161	1.022	1.043	1.064	1.084	1.104	1.123	1.142	1.161	1.179	1.197	1.214	1.231	1.248	1.264	1.280
162	1.026	1.048	1.069	1.089	1.109	1.128	1.147	1.166	1.184	1.202	1.220	1.237	1.253	1.270	1.286
163	1.031	1.053	1.074	1.094	1.114	1.133	1.152	1.171	1.190	1.208	1.225	1.242	1.259	1.276	1.292
164	1.036	1.057	1.078	1.099	1.119	1.138	1.157	1.176	1.195	1.213	1.230	1.247	1.264	1.281	1.297
165	1.040	1.062	1.083	1.104	1.124	1.144	1.163	1.182	1.200	1.218	1.236	1.253	1.270	1.287	1.303
166	1.045	1.066	1.088	1.109	1.129	1.149	1.168	1.187	1.205	1.223	1.241	1.259	1.276	1.293	1.309
167	1.049	1.071	1.092	1.113	1.134	1.154	1.173	1.192	1.210	1.229	1.247	1.264	1.281	1.298	1.315
168	1.054	1.076	1.097	1.118	1.139	1.159	1.178	1.197	1.216	1.234	1.252	1.270	1.287	1.304	1.320
169	1.058	1.080	1.102	1.123	1.143	1.163	1.183	1.202	1.221	1.239	1.257	1.275	1.292	1.309	1.326
170	1.063	1.085	1.107	1.128	1.148	1.168	1.188	1.207	1.226	1.245	1.263	1.281	1.298	1.315	1.332
171	1.067	1.089	1.111	1.132	1.153	1.173	1.193	1.212	1.231	1.250	1.268	1.286	1.303	1.320	1.337
172	1.072	1.094	1.116	1.137	1.158	1.178	1.198	1.218	1.237	1.255	1.273	1.291	1.309	1.326	1.343
173	1.076	1.098	1.120	1.141	1.162	1.183	1.203	1.223	1.242	1.260	1.278	1.296	1.314	1.331	1.348
174	1.080	1.103	1.125	1.146	1.167	1.188	1.208	1.228	1.247	1.265	1.284	1.302	1.320	1.337	1.354

TABLE II

SURFACE AREA IN SQUARE METERS (DU BOIS FORMULA). 35 KG. TO 49 KG., 120 CM. TO 184 CM.

	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49
120	1.047	1.060	1.073	1.085	1.097	1.109	1.120	1.132	1.143	1.155	1.166	1.176	1.187	1.198	1.208
121	1.053	1.066	1.079	1.091	1.103	1.115	1.127	1.139	1.150	1.161	1.172	1.183	1.194	1.205	1.216
122	1.060	1.073	1.085	1.098	1.110	1.122	1.134	1.146	1.157	1.168	1.180	1.191	1.201	1.212	1.223
123	1.066	1.079	1.092	1.104	1.117	1.129	1.141	1.152	1.164	1.175	1.187	1.198	1.209	1.220	1.230
124	1.072	1.085	1.098	1.111	1.123	1.135	1.147	1.159	1.171	1.182	1.194	1.205	1.216	1.227	1.237
125	1.079	1.092	1.105	1.117	1.130	1.142	1.154	1.166	1.178	1.189	1.201	1.212	1.223	1.234	1.245
126	1.085	1.098	1.111	1.124	1.137	1.149	1.161	1.173	1.184	1.196	1.208	1.219	1.230	1.241	1.252
127	1.091	1.104	1.117	1.130	1.143	1.156	1.168	1.180	1.191	1.203	1.215	1.226	1.237	1.248	1.259
128	1.097	1.111	1.124	1.137	1.150	1.162	1.174	1.186	1.198	1.210	1.221	1.233	1.244	1.255	1.266
129	1.104	1.117	1.130	1.143	1.156	1.168	1.181	1.193	1.205	1.217	1.228	1.240	1.251	1.262	1.274
130	1.110	1.123	1.137	1.150	1.163	1.175	1.187	1.199	1.211	1.223	1.235	1.247	1.258	1.269	1.281
131	1.116	1.130	1.143	1.156	1.169	1.182	1.194	1.206	1.218	1.230	1.242	1.254	1.265	1.277	1.288
132	1.122	1.136	1.149	1.162	1.175	1.188	1.200	1.213	1.225	1.237	1.249	1.261	1.272	1.284	1.295
133	1.128	1.142	1.156	1.169	1.182	1.195	1.207	1.220	1.232	1.244	1.256	1.268	1.279	1.291	1.302
134	1.134	1.148	1.162	1.175	1.188	1.201	1.213	1.226	1.238	1.250	1.262	1.274	1.286	1.298	1.309
135	1.141	1.155	1.168	1.181	1.194	1.207	1.220	1.233	1.245	1.257	1.269	1.281	1.293	1.305	1.316
136	1.147	1.161	1.174	1.188	1.201	1.214	1.227	1.239	1.252	1.264	1.276	1.288	1.300	1.312	1.323
137	1.153	1.167	1.181	1.194	1.207	1.220	1.233	1.246	1.258	1.271	1.283	1.295	1.307	1.319	1.330
138	1.159	1.173	1.187	1.200	1.214	1.227	1.240	1.253	1.265	1.278	1.290	1.302	1.314	1.326	1.337
139	1.165	1.179	1.193	1.207	1.220	1.233	1.246	1.260	1.272	1.284	1.297	1.309	1.321	1.333	1.344
140	1.171	1.185	1.199	1.213	1.227	1.240	1.253	1.266	1.278	1.291	1.304	1.316	1.327	1.339	1.351
141	1.177	1.192	1.206	1.219	1.233	1.246	1.259	1.272	1.285	1.298	1.310	1.323	1.334	1.346	1.358
142	1.183	1.198	1.212	1.225	1.239	1.253	1.266	1.279	1.292	1.304	1.317	1.329	1.341	1.353	1.365
143	1.189	1.204	1.218	1.232	1.246	1.259	1.272	1.285	1.298	1.311	1.323	1.336	1.348	1.360	1.372
144	1.195	1.210	1.224	1.238	1.252	1.265	1.278	1.292	1.305	1.318	1.330	1.343	1.355	1.367	1.379
145	1.201	1.216	1.230	1.244	1.258	1.272	1.285	1.298	1.311	1.324	1.337	1.350	1.362	1.374	1.386
146	1.207	1.222	1.237	1.251	1.265	1.278	1.292	1.305	1.318	1.331	1.344	1.356	1.369	1.381	1.393
147	1.213	1.228	1.243	1.257	1.271	1.284	1.298	1.311	1.324	1.338	1.350	1.363	1.375	1.388	1.400
148	1.219	1.234	1.249	1.263	1.277	1.291	1.304	1.318	1.331	1.344	1.357	1.370	1.382	1.395	1.407
149	1.225	1.240	1.255	1.269	1.283	1.297	1.311	1.324	1.338	1.351	1.364	1.377	1.389	1.402	1.414

150	1.231	1.246	1.261	1.275	1.289	1.303	1.317	1.331	1.344	1.357	1.370	1.383	1.396	1.408	1.421
151	1.237	1.252	1.267	1.281	1.296	1.310	1.323	1.337	1.351	1.364	1.377	1.390	1.402	1.415	1.428
152	1.243	1.258	1.273	1.287	1.302	1.316	1.329	1.343	1.357	1.370	1.383	1.396	1.409	1.422	1.434
153	1.249	1.264	1.279	1.294	1.308	1.322	1.336	1.350	1.363	1.377	1.390	1.403	1.416	1.429	1.441
154	1.255	1.270	1.285	1.300	1.314	1.328	1.342	1.356	1.370	1.383	1.396	1.409	1.422	1.435	1.448
155	1.261	1.276	1.291	1.306	1.321	1.335	1.349	1.363	1.376	1.390	1.403	1.416	1.429	1.442	1.455
156	1.267	1.282	1.297	1.312	1.327	1.341	1.355	1.369	1.383	1.396	1.410	1.423	1.436	1.449	1.462
157	1.273	1.288	1.303	1.318	1.333	1.347	1.361	1.375	1.389	1.403	1.416	1.429	1.442	1.455	1.468
158	1.279	1.294	1.309	1.324	1.339	1.354	1.368	1.382	1.396	1.410	1.423	1.436	1.449	1.462	1.475
159	1.285	1.300	1.315	1.330	1.345	1.360	1.374	1.388	1.402	1.416	1.429	1.443	1.456	1.469	1.482
160	1.291	1.306	1.321	1.336	1.351	1.366	1.380	1.395	1.409	1.423	1.436	1.450	1.463	1.476	1.489
161	1.296	1.312	1.327	1.342	1.357	1.372	1.386	1.401	1.415	1.429	1.442	1.456	1.469	1.482	1.495
162	1.302	1.318	1.333	1.348	1.363	1.378	1.393	1.407	1.421	1.435	1.449	1.463	1.476	1.489	1.502
163	1.308	1.324	1.339	1.354	1.369	1.384	1.399	1.414	1.428	1.442	1.455	1.469	1.483	1.496	1.509
164	1.313	1.329	1.345	1.360	1.375	1.390	1.405	1.420	1.434	1.448	1.462	1.475	1.489	1.502	1.516
165	1.319	1.335	1.351	1.366	1.382	1.397	1.411	1.426	1.440	1.454	1.468	1.482	1.496	1.509	1.522
166	1.325	1.341	1.357	1.372	1.388	1.403	1.417	1.432	1.446	1.461	1.475	1.489	1.502	1.516	1.529
167	1.331	1.347	1.363	1.378	1.394	1.409	1.423	1.438	1.452	1.467	1.481	1.495	1.508	1.522	1.536
168	1.337	1.353	1.369	1.384	1.400	1.415	1.430	1.444	1.459	1.473	1.487	1.501	1.515	1.529	1.542
169	1.342	1.359	1.375	1.390	1.406	1.421	1.436	1.451	1.465	1.480	1.494	1.508	1.522	1.535	1.548
170	1.348	1.364	1.380	1.396	1.412	1.427	1.442	1.457	1.471	1.486	1.500	1.514	1.528	1.542	1.555
171	1.354	1.370	1.386	1.402	1.418	1.433	1.448	1.463	1.478	1.492	1.507	1.521	1.535	1.548	1.562
172	1.360	1.376	1.392	1.408	1.424	1.439	1.454	1.469	1.484	1.499	1.513	1.527	1.541	1.555	1.569
173	1.365	1.382	1.398	1.414	1.430	1.445	1.460	1.476	1.490	1.505	1.519	1.533	1.547	1.561	1.575
174	1.371	1.388	1.404	1.420	1.436	1.451	1.467	1.482	1.497	1.511	1.526	1.540	1.554	1.568	1.582
175	1.377	1.393	1.410	1.426	1.442	1.457	1.473	1.488	1.503	1.518	1.532	1.547	1.561	1.575	1.589
176	1.383	1.399	1.416	1.432	1.448	1.464	1.479	1.494	1.509	1.524	1.539	1.553	1.567	1.581	1.595
177	1.388	1.405	1.422	1.438	1.454	1.470	1.485	1.500	1.515	1.530	1.545	1.560	1.574	1.588	1.602
178	1.394	1.411	1.428	1.444	1.460	1.476	1.491	1.507	1.522	1.537	1.552	1.566	1.581	1.595	1.609
179	1.400	1.417	1.434	1.450	1.466	1.482	1.497	1.513	1.528	1.543	1.558	1.573	1.587	1.601	1.615
180	1.405	1.422	1.439	1.455	1.471	1.487	1.503	1.518	1.534	1.549	1.564	1.579	1.593	1.607	1.621
181	1.411	1.428	1.445	1.461	1.477	1.493	1.509	1.525	1.540	1.555	1.570	1.585	1.599	1.614	1.628
182	1.417	1.434	1.451	1.467	1.483	1.499	1.515	1.531	1.546	1.561	1.576	1.591	1.606	1.620	1.635
183	1.422	1.439	1.456	1.473	1.489	1.505	1.521	1.537	1.552	1.567	1.582	1.597	1.612	1.626	1.641
184	1.428	1.445	1.462	1.479	1.495	1.511	1.527	1.543	1.558	1.574	1.589	1.604	1.618	1.633	1.647

TABLE III

SURFACE AREA IN SQUARE METERS (DU BOIS FORMULA). 50 KG. TO 64 KG., 135 CM. TO 199 CM.

	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64
135	1.327	1.338	1.349	1.361	1.372	1.383	1.393	1.404	1.414	1.424	1.434	1.445	1.455	1.465	1.475
136	1.335	1.346	1.357	1.368	1.379	1.390	1.401	1.411	1.421	1.432	1.442	1.452	1.463	1.472	1.482
137	1.342	1.353	1.364	1.375	1.386	1.397	1.408	1.419	1.429	1.439	1.450	1.460	1.470	1.480	1.490
138	1.349	1.360	1.371	1.383	1.394	1.405	1.415	1.426	1.436	1.447	1.457	1.468	1.478	1.488	1.498
139	1.356	1.367	1.378	1.390	1.401	1.412	1.423	1.434	1.444	1.455	1.465	1.476	1.486	1.496	1.506
140	1.363	1.374	1.385	1.397	1.408	1.419	1.430	1.441	1.451	1.462	1.473	1.483	1.493	1.503	1.514
141	1.370	1.381	1.393	1.404	1.416	1.427	1.438	1.449	1.459	1.470	1.480	1.491	1.501	1.511	1.522
142	1.377	1.388	1.400	1.412	1.423	1.434	1.445	1.456	1.467	1.477	1.488	1.499	1.509	1.519	1.529
143	1.384	1.395	1.407	1.419	1.430	1.441	1.452	1.463	1.474	1.485	1.496	1.506	1.517	1.527	1.537
144	1.391	1.402	1.414	1.426	1.437	1.449	1.460	1.471	1.482	1.492	1.503	1.514	1.524	1.535	1.545
145	1.398	1.410	1.421	1.433	1.445	1.456	1.467	1.478	1.489	1.500	1.511	1.522	1.532	1.542	1.553
146	1.405	1.417	1.428	1.440	1.452	1.464	1.475	1.486	1.497	1.508	1.518	1.529	1.540	1.550	1.561
147	1.412	1.424	1.435	1.447	1.459	1.471	1.482	1.493	1.504	1.515	1.526	1.537	1.547	1.558	1.568
148	1.419	1.431	1.443	1.455	1.467	1.478	1.489	1.500	1.511	1.523	1.534	1.545	1.555	1.566	1.576
149	1.426	1.438	1.450	1.462	1.474	1.485	1.497	1.508	1.519	1.530	1.541	1.552	1.563	1.573	1.584
150	1.433	1.445	1.457	1.469	1.481	1.492	1.504	1.515	1.526	1.537	1.548	1.559	1.570	1.581	1.591
151	1.440	1.452	1.464	1.476	1.488	1.500	1.511	1.523	1.534	1.545	1.556	1.567	1.578	1.589	1.599
152	1.447	1.459	1.471	1.483	1.495	1.507	1.518	1.530	1.541	1.552	1.563	1.574	1.585	1.596	1.607
153	1.454	1.466	1.478	1.490	1.502	1.514	1.525	1.537	1.548	1.560	1.571	1.582	1.593	1.604	1.614
154	1.461	1.473	1.485	1.497	1.509	1.521	1.532	1.544	1.555	1.567	1.578	1.589	1.600	1.611	1.622
155	1.468	1.480	1.492	1.504	1.516	1.528	1.540	1.552	1.563	1.574	1.586	1.597	1.608	1.619	1.630
156	1.474	1.486	1.498	1.511	1.523	1.535	1.547	1.559	1.570	1.582	1.593	1.604	1.615	1.626	1.637
157	1.481	1.493	1.505	1.518	1.530	1.542	1.554	1.566	1.577	1.589	1.600	1.612	1.623	1.634	1.645
158	1.488	1.500	1.512	1.525	1.538	1.550	1.562	1.573	1.585	1.596	1.608	1.620	1.631	1.642	1.653
159	1.495	1.507	1.519	1.532	1.545	1.557	1.569	1.581	1.592	1.604	1.615	1.627	1.638	1.649	1.660
160	1.502	1.514	1.526	1.539	1.552	1.564	1.576	1.588	1.599	1.611	1.623	1.635	1.646	1.657	1.668
161	1.508	1.521	1.533	1.546	1.559	1.571	1.583	1.595	1.606	1.618	1.630	1.641	1.653	1.664	1.675
162	1.515	1.528	1.540	1.553	1.566	1.578	1.590	1.602	1.614	1.626	1.637	1.649	1.660	1.671	1.683
163	1.522	1.535	1.547	1.560	1.573	1.585	1.597	1.609	1.621	1.633	1.645	1.657	1.668	1.679	1.690
164	1.529	1.541	1.554	1.567	1.580	1.592	1.604	1.616	1.628	1.640	1.652	1.664	1.675	1.686	1.697

165	1.535	1.548	1.561	1 574	1.587	1.599	1.611	1.624	1.635	1.647	1.659	1.671	1.683	1.694	1.705
166	1.542	1.555	1.568	1.581	1.594	1.606	1.618	1.631	1.642	1.654	1.666	1.678	1.690	1.701	1.713
167	1.549	1.561	1.574	1.587	1.600	1.613	1.625	1.638	1.649	1.661	1.673	1.685	1.697	1.708	1.720
168	1.555	1.568	1.581	1.594	1.607	1 620	1.632	1.645	1.657	1.669	1.681	1.693	1.705	1.716	1.727
169	1.562	1.575	1.588	1.601	1.614	1.627	1.639	1.652	1.664	1.676	1.688	1.700	1.712	1.723	1.735
170	1.569	1.582	1.595	1 608	1.621	1.634	1.646	1.659	1.671	1.683	1.695	1.707	1.719	1.731	1.742
171	1.576	1.589	1.602	1.615	1.628	1.641	1.653	1.666	1.678	1.690	1.703	1.715	1.727	1.738	1.750
172	1.583	1.596	1.609	1.622	1.635	1.648	1.660	1 673	1.686	1.698	1.710	1.722	1.734	1.745	1.757
173	1.589	1.602	1.615	1.628	1.642	1.655	1.667	1.680	1.693	1.705	1.717	1.729	1.741	1.753	1.765
174	1.596	1.609	1.622	1.635	1.649	1.662	1.675	1.687	1.699	1.712	1.724	1.737	1.749	1.760	1.772
175	1.603	1.616	1.629	1.642	1.656	1.669	1.682	1.694	1.706	1.719	1.731	1.744	1.756	1.768	1.780
176	1.609	1.622	1.635	1.649	1.663	1.676	1.689	1.701	1.713	1.726	1.739	1.751	1 763	1.775	1.787
177	1.616	1.629	1.642	1.656	1.670	1.683	1.696	1.708	1.720	1.733	1.746	1.758	1.771	1.782	1.794
178	1.623	1.636	1.649	1.663	1.677	1.690	1 703	1.715	1.728	1.740	1.753	1.766	1.778	1.790	1.802
179	1.629	1.642	1.656	1.670	1.683	1.697	1.710	1.722	1.735	1.747	1 760	1.773	1.785	1.797	1.809
180	1.635	1.649	1.662	1.676	1.690	1.703	1.716	1.729	1.742	1.754	1 767	1 780	1.792	1.804	1.816
181	1.642	1.655	1.669	1.683	1.697	1.710	1.723	1.736	1.749	1.762	1.774	1.787	1.799	1.811	1.823
182	1.649	1.662	1.676	1.690	1.704	1.717	1.730	1.743	1 756	1.769	1.781	1.794	1.806	1.819	1.831
183	1.655	1.668	1.682	1.696	1.710	1.723	1.737	1 750	1.763	1.776	1.788	1.801	1.813	1.826	1.838
184	1.661	1.675	1.689	1.703	1.717	1 730	1.744	1.757	1.770	1.783	1.796	1.808	1.821	1.833	1.845
185	1.668	1.682	1.696	1.710	1.724	1.737	1.751	1.764	1.777	1.790	1.803	1.816	1.828	1.840	1.853
186	1.675	1.688	1.702	1.717	1.731	1.744	1.758	1 771	1.784	1.797	1.810	1.823	1.835	1.848	1.860
187	1.681	1.695	1.709	1.723	1.737	1.751	1.764	1.778	1.791	1.804	1.817	1.830	1.842	1.855	1.867
188	1.688	1.702	1.716	1.730	1.744	1.758	1.771	1.785	1.798	1.811	1.824	1.837	1.849	1.862	1.875
189	1.695	1.708	1.722	1.737	1.751	1.765	1.778	1.792	1.805	1.818	1.831	1.844	1.856	1.869	1.882
190	1.701	1.715	1.729	1.743	1.758	1.771	1.785	1.798	1.811	1.825	1.838	1.851	1.863	1.876	1.889
191	1.707	1.721	1.735	1.750	1.764	1.778	1.791	1.805	1.818	1.832	1.845	1.858	1.870	1.883	1.896
192	1.714	1.728	1.742	1.757	1.771	1.785	1.798	1.812	1.825	1.839	1.852	1.865	1.878	1.891	1.903
193	1.721	1.735	1.749	1.764	1.778	1.792	1.805	1.819	1.832	1.846	1.859	1.872	1.885	1.898	1.911
194	1.727	1.741	1.755	1.770	1.784	1.798	1.812	1.826	1.839	1.853	1.866	1.879	1.892	1.905	1.918
195	1.733	1.747	1.762	1.777	1.791	1.805	1.819	1.833	1.846	1.860	1.873	1.886	1.899	1.912	1.925
196	1.740	1.754	1.768	1.783	1.798	1.812	1.826	1.840	1.853	1.866	1.880	1.893	1.906	1.919	1.832
197	1.746	1.760	1.775	1.790	1.804	1.818	1.832	1.846	1.860	1.873	1.887	1.900	1.913	1.926	1.939
198	1.752	1.767	1.781	1.796	1.811	1.825	1.839	1.853	1.866	1.880	1.894	1.907	1.920	1.933	1.946
199	1.758	1.773	1.788	1.803	1.818	1.832	1.846	1.860	1.873	1.887	1.900	1.913	1.926	1.940	1.953

TABLE IV

SURFACE AREA IN SQUARE METERS (DU BOIS FORMULA). 65 KG. TO 79 KG., 135 CM. TO 199 CM.

	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79
135	1.484	1.494	1.503	1.513	1.522	1.532	1.541	1.550	1.559	1.568	1.577	1.586	1.595	1.604	1.613
136	1.492	1.502	1.511	1.521	1.530	1.540	1.549	1.558	1.567	1.577	1.586	1.595	1.604	1.612	1.621
137	1.500	1.510	1.519	1.529	1.539	1.548	1.557	1.567	1.576	1.585	1.594	1.603	1.612	1.621	1.630
138	1.508	1.518	1.528	1.537	1.547	1.556	1.566	1.575	1.584	1.593	1.603	1.611	1.620	1.629	1.638
139	1.516	1.526	1.536	1.545	1.555	1.565	1.574	1.583	1.593	1.602	1.611	1.620	1.629	1.638	1.647
140	1.524	1.534	1.544	1.553	1.563	1.573	1.582	1.591	1.601	1.610	1.619	1.628	1.637	1.646	1.655
141	1.532	1.542	1.552	1.561	1.571	1.581	1.590	1.600	1.609	1.618	1.628	1.637	1.646	1.655	1.664
142	1.540	1.550	1.560	1.569	1.579	1.589	1.598	1.608	1.617	1.627	1.636	1.645	1.654	1.663	1.673
143	1.547	1.557	1.567	1.577	1.587	1.597	1.607	1.616	1.625	1.635	1.644	1.653	1.663	1.672	1.681
144	1.555	1.565	1.575	1.585	1.595	1.605	1.615	1.624	1.634	1.643	1.653	1.662	1.671	1.680	1.690
145	1.563	1.573	1.583	1.593	1.603	1.613	1.623	1.633	1.642	1.652	1.661	1.670	1.680	1.689	1.698
146	1.571	1.581	1.591	1.601	1.611	1.621	1.631	1.641	1.650	1.660	1.670	1.679	1.688	1.697	1.707
147	1.579	1.589	1.599	1.609	1.619	1.629	1.639	1.649	1.658	1.668	1.678	1.687	1.697	1.706	1.715
148	1.587	1.597	1.607	1.617	1.627	1.637	1.647	1.657	1.667	1.676	1.686	1.696	1.705	1.714	1.724
149	1.594	1.605	1.615	1.625	1.635	1.645	1.655	1.665	1.675	1.685	1.694	1.704	1.714	1.723	1.732
150	1.602	1.613	1.623	1.633	1.643	1.653	1.663	1.673	1.683	1.693	1.703	1.712	1.722	1.731	1.741
151	1.610	1.620	1.631	1.641	1.651	1.661	1.671	1.681	1.691	1.701	1.711	1.720	1.730	1.739	1.749
152	1.617	1.628	1.638	1.649	1.659	1.669	1.679	1.689	1.699	1.709	1.719	1.728	1.738	1.747	1.757
153	1.625	1.636	1.646	1.657	1.667	1.677	1.687	1.697	1.707	1.717	1.727	1.737	1.747	1.756	1.766
154	1.633	1.643	1.654	1.664	1.675	1.685	1.695	1.705	1.715	1.725	1.735	1.745	1.755	1.764	1.774
155	1.641	1.651	1.662	1.672	1.683	1.693	1.703	1.713	1.723	1.733	1.743	1.753	1.763	1.773	1.782
156	1.648	1.659	1.670	1.680	1.690	1.701	1.711	1.721	1.731	1.741	1.751	1.761	1.771	1.781	1.791
157	1.656	1.666	1.677	1.688	1.698	1.709	1.719	1.729	1.739	1.749	1.760	1.770	1.780	1.789	1.799
158	1.664	1.675	1.685	1.696	1.706	1.717	1.727	1.737	1.747	1.758	1.768	1.778	1.788	1.798	1.808
159	1.671	1.682	1.693	1.703	1.714	1.725	1.735	1.745	1.755	1.766	1.776	1.786	1.796	1.806	1.816
160	1.679	1.690	1.701	1.711	1.722	1.733	1.743	1.753	1.763	1.774	1.784	1.794	1.804	1.814	1.824
161	1.686	1.697	1.708	1.719	1.729	1.740	1.751	1.761	1.771	1.782	1.792	1.802	1.812	1.822	1.832
162	1.694	1.705	1.716	1.727	1.737	1.748	1.759	1.769	1.780	1.790	1.800	1.810	1.820	1.830	1.840
163	1.702	1.713	1.724	1.735	1.745	1.756	1.767	1.777	1.788	1.798	1.808	1.818	1.829	1.839	18.49
164	1.709	1.720	1.731	1.742	1.753	1.764	1.774	1.785	1.795	1.806	1.816	1.826	1.837	1.847	1.857

165	1.717	1.728	1.739	1.750	1.761	1.772	1.782	1.793	1.803	1.814	1.824	1.834	1.845	1.855	1.865
166	1.724	1.735	1.747	1.758	1.769	1.780	1.790	1.801	1.811	1.822	1.832	1.842	1.853	1.863	1.873
167	1.731	1.743	1.754	1.765	1.776	1.787	1.798	1.808	1.819	1.829	1.840	1.850	1.861	1.871	1.881
168	1.739	1.750	1.762	1.773	1.784	1.795	1.806	1.816	1.827	1.837	1.848	1.858	1.869	1.879	1.889
169	1.747	1.758	1.769	1.780	1.791	1.802	1.813	1.824	1.835	1.845	1.856	1.866	1.877	1.887	1.897
170	1.754	1.765	1.777	1.788	1.799	1.810	1.821	1.832	1.843	1.853	1.864	1.874	1.885	1.895	1.905
171	1.762	1.773	1.785	1.796	1.807	1.818	1.829	1.840	1.851	1.861	1.872	1.882	1.893	1.903	1.914
172	1.769	1.781	1.792	1.803	1.814	1.826	1.837	1.848	1.859	1.869	1.880	1.890	1.901	1.912	1.922
173	1.777	1.788	1.799	1.810	1.821	1.833	1.844	1.855	1.866	1.877	1.888	1.898	1.909	1.920	1.930
174	1.784	1.796	1.807	1.818	1.829	1.841	1.852	1.863	1.874	1.885	1.896	1.906	1.917	1.928	1.938
175	1.791	1.803	1.815	1.826	1.837	1.849	1.860	1.871	1.882	1.893	1.904	1.914	1.925	1.936	1.946
176	1.799	1.811	1.822	1.834	1.845	1.857	1.868	1.879	1.890	1.901	1.912	1.922	1.933	1.944	1.954
177	1.806	1.818	1.830	1.841	1.853	1.864	1.876	1.887	1.898	1.909	1.920	1.930	1.941	1.952	1.962
178	1.814	1.826	1.838	1.849	1.861	1.872	1.884	1.895	1.906	1.917	1.928	1.938	1.949	1.960	1.971
179	1.821	1.833	1.845	1.857	1.868	1.880	1.891	1.902	1.913	1.924	1.935	1.946	1.957	1.968	1.979
180	1.828	1.840	1.852	1.864	1.875	1.887	1.898	1.909	1.920	1.932	1.943	1.954	1.965	1.975	1.986
181	1.836	1.848	1.860	1.871	1.883	1.895	1.906	1.917	1.928	1.940	1.951	1.962	1.973	1.983	1.994
182	1.843	1.855	1.867	1.879	1.891	1.902	1.914	1.925	1.936	1.948	1.959	1.970	1.981	1.991	2.002
183	1.850	1.862	1.874	1.886	1.898	1.909	1.921	1.932	1.944	1.955	1.966	1.977	1.988	1.999	2.010
184	1.858	1.870	1.882	1.894	1.905	1.917	1.929	1.940	1.952	1.963	1.974	1.985	1.996	2.007	2.018
185	1.865	1.877	1.889	1.901	1.913	1.925	1.936	1.948	1.959	1.971	1.982	1.993	2.004	2.015	2.026
186	1.872	1.885	1.897	1.909	1.921	1.932	1.944	1.956	1.967	1.978	1.990	2.001	2.012	2.023	2.034
187	1.879	1.892	1.904	1.916	1.928	1.940	1.951	1.963	1.974	1.986	1.997	2.008	2.020	2.031	2.042
188	1.887	1.899	1.912	1.924	1.936	1.948	1.959	1.971	1.982	1.994	2.005	2.016	2.028	2.039	2.050
189	1.895	1.907	1.919	1.931	1.943	1.955	1.967	1.979	1.990	2.002	2.013	2.024	2.036	2.047	2.058
190	1.902	1.914	1.926	1.938	1.950	1.962	1.974	1.986	1.998	2.009	2.021	2.032	2.044	2.055	2.066
191	1.909	1.921	1.933	1.945	1.957	1.970	1.982	1.994	2.005	2.017	2.028	2.039	2.051	2.062	2.074
192	1.916	1.928	1.941	1.953	1.965	1.977	1.989	2.001	2.013	2.024	2.036	2.047	2.059	2.070	2.082
193	1.924	1.936	1.948	1.961	1.973	1.985	1.997	2.009	2.021	2.032	2.044	2.055	2.067	2.078	2.090
194	1.931	1.943	1.956	1.968	1.980	1.992	2.004	2.016	2.028	2.040	2.052	2.063	2.075	2.086	2.097
195	1.938	1.950	1.963	1.975	1.987	2.000	2.012	2.024	2.036	2.047	2.059	2.071	2.083	2.094	2.105
196	1.945	1.957	1.970	1.982	1.995	2.007	2.019	2.031	2.043	2.055	2.067	2.078	2.090	2.101	2.113
197	1.952	1.965	1.977	1.990	2.002	2.015	2.027	2.039	2.051	2.063	2.074	2.086	2.097	2.109	2.121
198	1.959	1.972	1.985	1.997	2.010	2.022	2.034	2.046	2.058	2.070	2.082	2.094	2.105	2.117	2.129
199	1.966	1.979	1.992	2.004	2.017	2.030	2.042	2.054	2.066	2.078	2.090	2.101	2.113	2.125	2.137

TABLE V

SURFACE AREA IN SQUARE METERS (DU BOIS FORMULA). 80 KG. TO 94 KG., 135 CM. TO 199 CM.

	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94
135	1.621	1.629	1.638	1.647	1.655	1.664	1.672	1.680	1.688	1.696	1.704	1.712	1.720	1.728	1.736
136	1.630	1.638	1.647	1.655	1.664	1.673	1.681	1.689	1.697	1.705	1.713	1.721	1.729	1.737	1.745
137	1.638	1.646	1.655	1.664	1.673	1.682	1.690	1.698	1.706	1.714	1.722	1.731	1.739	1.747	1.755
138	1.647	1.655	1.664	1.673	1.682	1.690	1.699	1.707	1.715	1.723	1.731	1.740	1.748	1.756	1.764
139	1.656	1.664	1.673	1.682	1.690	1.699	1.708	1.716	1.724	1.733	1.741	1.749	1.757	1.765	1.773
140	1.664	1.672	1.681	1.690	1.699	1.708	1.716	1.725	1.733	1.742	1.750	1.758	1.766	1.774	1.782
141	1.673	1.681	1.690	1.699	1.708	1.717	1.725	1.734	1.742	1.751	1.759	1.767	1.775	1.784	1.792
142	1.682	1.690	1.699	1.708	1.717	1.726	1.734	1.743	1.751	1.760	1.768	1.776	1.784	1.793	1.801
143	1.690	1.699	1.708	1.717	1.725	1.735	1.743	1.751	1.760	1.769	1.777	1.785	1.793	1.802	1.810
144	1.699	1.707	1.716	1.725	1.734	1.743	1.752	1.760	1.769	1.778	1.786	1.794	1.803	1.811	1.819
145	1.707	1.716	1.725	1.734	1.743	1.752	1.761	1.769	1.778	1.787	1.795	1.803	1.812	1.820	1.828
146	1.716	1.725	1.734	1.743	1.752	1.761	1.770	1.778	1.787	1.796	1.804	1.813	1.821	1.829	1.837
147	1.724	1.733	1.742	1.751	1.760	1.770	1.778	1.787	1.796	1.805	1.813	1.822	1.830	1.838	1.846
148	1.733	1.742	1.751	1.760	1.769	1.779	1.787	1.796	1.805	1.814	1.822	1.831	1.839	1.848	1.856
149	1.741	1.750	1.760	1.769	1.778	1.787	1.796	1.805	1.813	1.822	1.831	1.840	1.848	1.857	1.865
150	1.750	1.759	1.768	1.777	1.786	1.796	1.805	1.814	1.822	1.831	1.840	1.848	1.857	1.866	1.874
151	1.758	1.767	1.777	1.786	1.795	1.805	1.814	1.823	1.831	1.840	1.848	1.857	1.866	1.875	1.883
152	1.766	1.775	1.785	1.794	1.803	1.813	1.822	1.831	1.840	1.849	1.857	1.866	1.875	1.883	1.892
153	1.775	1.784	1.794	1.803	1.812	1.822	1.831	1.840	1.848	1.857	1.866	1.875	1.884	1.892	1.901
154	1.783	1.792	1.802	1.811	1.821	1.830	1.839	1.848	1.857	1.866	1.875	1.884	1.893	1.901	1.910
155	1.792	1.801	1.811	1.820	1.829	1.839	1.848	1.857	1.866	1.875	1.884	1.893	1.902	1.910	1.919
156	1.800	1.809	1.819	1.829	1.838	1.848	1.857	1.866	1.875	1.884	1.893	1.902	1.910	1.919	1.928
157	1.808	1.818	1.828	1.837	1.846	1.856	1.865	1.874	1.883	1.892	1.901	1.910	1.919	1.928	1.937
158	1.817	1.827	1.837	1.846	1.855	1.865	1.874	1.883	1.892	1.901	1.910	1.919	1.928	1.937	1.946
159	1.825	1.835	1.845	1.854	1.863	1.873	1.882	1.891	1.901	1.910	1.919	1.928	1.937	1.946	1.955
160	1.834	1.844	1.854	1.863	1.872	1.882	1.891	1.900	1.910	1.919	1.928	1.937	1.946	1.955	1.964
161	1.842	1.852	1.862	1.871	1.880	1.890	1.899	1.909	1.918	1.927	1.936	1.945	1.954	1.963	1.972
162	1.850	1.860	1.870	1.879	1.889	1.899	1.908	1.917	1.927	1.936	1.945	1.954	1.963	1.972	1.981
163	1.859	1.868	1.878	1.888	1.898	1.908	1.917	1.926	1.936	1.945	1.954	1.963	1.972	1.981	1.990
164	1.867	1.876	1.886	1.896	1.906	1.916	1.925	1.934	1.944	1.953	1.962	1.972	1.981	1.990	1.999

165	1.875	1.885	1.895	1.905	1.914	1.924	1.934	1.943	1.953	1.962	1.971	1.981	1.990	1.999	2.008
166	1.883	1.893	1.903	1.913	1.923	1.933	1.942	1.952	1.961	1.971	1.980	1.989	1.998	2.008	2.017
167	1.891	1.901	1.911	1.921	1.931	1.941	1.950	1.960	1.969	1.979	1.988	1.998	2.007	2.016	2.025
168	1.899	1.909	1.920	1.930	1.939	1.949	1.959	1.968	1.978	1.988	1.997	2.006	2.016	2.025	2.034
169	1.908	1.918	1.928	1.938	1.948	1.958	1.967	1.977	1.987	1.996	2.005	2.015	2.024	2.034	2.043
170	1.916	1.926	1.936	1.946	1.956	1.966	1.976	1.985	1.995	2.005	2.014	2.023	2.033	2.042	2.051
171	1.924	1.934	1.944	1.954	1.964	1.974	1.984	1.994	2.004	2.014	2.023	2.032	2.042	2.051	2.060
172	1.932	1.942	1.953	1.963	1.973	1.983	1.993	2.003	2.012	2.022	2.031	2.041	2.051	2.060	2.069
173	1.940	1.950	1.961	1.971	1.981	1.991	2.001	2.011	2.020	2.030	2.039	2.049	2.059	2.068	2.077
174	1.948	1.958	1.969	1.979	1.989	2.000	2.010	2.019	2.029	2.039	2.048	2.058	2.068	2.077	2.086
175	1.957	1.967	1.977	1.987	1.998	2.008	2.018	2.028	2.038	2.048	2.057	2.067	2.076	2.086	2.095
176	1.965	1.975	1.986	1.996	2.006	2.017	2.026	2.036	2.046	2.056	2.065	2.075	2.085	2.095	2.104
177	1.973	1.983	1.994	2.004	2.014	2.025	2.035	2.045	2.054	2.064	2.074	2.084	2.094	2.103	2.113
178	1.981	1.991	2.002	2.012	2.023	2.034	2.044	2.054	2.063	2.073	2.083	2.093	2.103	2.112	2.122
179	1.989	1.999	2.010	2.020	2.031	2.042	2.052	2.062	2.072	2.082	2.091	2.101	2.111	2.121	2.130
180	1.997	2.007	2.018	2.028	2.039	2.050	2.060	2.070	2.080	2.090	2.099	2.109	2.119	2.129	2.138
181	2.005	2.015	2.026	2.037	2.047	2.058	2.068	2.078	2.088	2.098	2.108	2.118	2.128	2.137	2.147
182	2.013	2.023	2.034	2.045	2.055	2.066	2.076	2.086	2.097	2.107	2.116	2.126	2.136	2.146	2.156
183	2.021	2.031	2.042	2.053	2.063	2.074	2.084	2.094	2.105	2.115	2.124	2.134	2.144	2.154	2.164
184	2.029	2.040	2.051	2.061	2.072	2.083	2.093	2.103	2.113	2.123	2.133	2.143	2.153	2.163	2.173
185	2.037	2.048	2.059	2.069	2.080	2.091	2.101	2.111	2.121	2.131	2.141	2.152	2.162	2.171	2.181
186	2.045	2.056	2.067	2.077	2.088	2.099	2.109	2.119	2.130	2.140	2.150	2.160	2.170	2.180	2.190
187	2.053	2.064	2.075	2.085	2.096	2.107	2.117	2.127	2.138	2.148	2.158	2.168	2.178	2.188	2.198
188	2.061	2.072	2.083	2.094	2.104	2.115	2.126	2.136	2.146	2.157	2.167	2.177	2.187	2.197	2.207
189	2.069	2.080	2.091	2.102	2.112	2.123	2.134	2.144	2.155	2.165	2.175	2.186	2.196	2.206	2.216
190	2.077	2.088	2.099	2.110	2.120	2.131	2.142	2.152	2.163	2.173	2.183	2.194	2.204	2.214	2.224
191	2.085	2.096	2.107	2.118	2.128	2.139	2.150	2.160	2.171	2.181	2.191	2.202	2.212	2.222	2.232
192	2.093	2.104	2.115	2.126	2.136	2.147	2.158	2.169	2.179	2.190	2.200	2.211	2.221	2.231	2.241
193	2.101	2.112	2.123	2.134	2.145	2.156	2.167	2.177	2.188	2.199	2.209	2.219	2.229	2.240	2.250
194	2.109	2.120	2.131	2.142	2.153	2.164	2.175	2.185	2.196	2.207	2.217	2.227	2.238	2.248	2.258
195	2.116	2.127	2.139	2.150	2.161	2.172	2.183	2.193	2.204	2.215	2.225	2.236	2.246	2.256	2.266
196	2.124	2.135	2.147	2.158	2.169	2.180	2.191	2.201	2.212	2.223	2.233	2.244	2.254	2.265	2.275
197	2.132	2.143	2.155	2.166	2.177	2.188	2.199	2.210	2.220	2.231	2.241	2.252	2.263	2.273	2.283
198	2.140	2.151	2.163	2.174	2.185	2.196	2.207	2.218	2.228	2.239	2.250	2.260	2.271	2.281	2.291
199	2.148	2.159	2.171	2.182	2.193	2.204	2.215	2.226	2.237	2.248	2.258	2.269	2.279	2.290	2.300

TABLE VI

SURFACE AREA IN SQUARE METERS (DU BOIS FORMULA). 95 KG. TO 109 KG., 135 CM. TO 199 CM.

	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109
135	1.744	1.752	1.760	1.767	1.775	1.782	1.790	1.798	1.805	1.812	1.820	1.827	1.834	1.842	1.849
136	1.753	1.761	1.769	1.777	1.784	1.792	1.799	1.807	1.814	1.822	1.829	1.837	1.844	1.851	1.859
137	1.763	1.771	1.778	1.786	1.793	1.801	1.809	1.817	1.824	1.831	1.839	1.847	1.854	1.861	1.869
138	1.772	1.780	1.788	1.796	1.803	1.811	1.818	1.826	1.834	1.841	1.849	1.857	1.864	1.871	1.878
139	1.781	1.789	1.797	1.805	1.813	1.821	1.828	1.836	1.843	1.851	1.859	1.866	1.874	1.881	1.888
140	1.790	1.798	1.806	1.814	1.822	1.830	1.837	1.845	1.853	1.860	1.868	1.876	1.883	1.891	1.898
141	1.800	1.808	1.816	1.824	1.831	1.840	1.847	1.855	1.863	1.870	1.878	1.886	1.893	1.901	1.908
142	1.809	1.817	1.825	1.833	1.841	1.849	1.857	1.865	1.872	1.880	1.888	1.895	1.903	1.910	1.918
143	1.818	1.826	1.834	1.842	1.850	1.858	1.866	1.874	1.881	1.889	1.897	1.905	1.912	1.920	1.928
144	1.827	1.836	1.844	1.852	1.860	1.868	1.875	1.884	1.891	1.899	1.907	1.915	1.922	1.930	1.937
145	1.837	1.845	1.853	1.861	1.869	1.877	1.885	1.893	1.901	1.909	1.916	1.924	1.932	1.940	1.947
146	1.846	1.854	1.863	1.871	1.879	1.887	1.895	1.903	1.910	1.918	1.926	1.934	1.942	1.949	1.957
147	1.855	1.863	1.872	1.880	1.888	1.896	1.904	1.912	1.920	1.928	1.936	1.944	1.951	1.959	1.967
148	1.864	1.873	1.881	1.889	1.897	1.905	1.913	1.921	1.929	1.937	1.945	1.953	1.961	1.969	1.977
149	1.873	1.882	1.890	1.898	1.906	1.915	1.923	1.931	1.939	1.947	1.955	1.963	1.971	1.979	1.986
150	1.882	1.891	1.899	1.908	1.916	1.924	1.932	1.940	1.948	1.956	1.964	1.972	1.980	1.988	1.996
151	1.891	1.900	1.909	1.917	1.925	1.933	1.941	1.949	1.957	1.966	1.974	1.982	1.990	1.998	2.006
152	1.900	1.909	1.918	1.926	1.934	1.942	1.950	1.959	1.967	1.975	1.983	1.991	1.999	2.007	2.015
153	1.909	1.918	1.927	1.935	1.943	1.952	1.960	1.968	1.976	1.984	1.993	2.001	2.009	2.017	2.024
154	1.918	1.927	1.936	1.944	1.952	1.961	1.969	1.977	1.985	1.993	2.002	2.010	2.018	2.026	2.034
155	1.927	1.936	1.945	1.953	1.962	1.970	1.978	1.987	1.995	2.003	2.011	2.020	2.028	2.036	2.044
156	1.936	1.945	1.954	1.962	1.971	1.979	1.987	1.996	2.004	2.012	2.021	2.029	2.037	2.045	2.053
157	1.945	1.954	1.963	1.972	1.980	1.988	1.997	2.005	2.013	2.022	2.030	2.038	2.046	2.054	2.063
158	1.955	1.963	1.972	1.981	1.989	1.998	2.006	2.015	2.023	2.031	2.040	2.048	2.056	2.064	2.073
159	1.964	1.972	1.981	1.990	1.998	2.007	2.015	2.024	2.032	2.040	2.049	2.057	2.065	2.073	2.082
160	1.973	1.981	1.990	1.999	2.007	2.016	2.024	2.033	2.042	2.050	2.059	2.067	2.075	2.083	2.091
161	1.981	1.990	1.999	2.008	2.016	2.025	2.033	2.042	2.051	2.059	2.068	2.076	2.084	2.092	2.100
162	1.990	1.999	2.008	2.017	2.025	2.034	2.043	2.052	2.060	2.068	2.077	2.085	2.094	2.102	2.110
163	1.999	2.009	2.017	2.026	2.034	2.043	2.052	2.061	2.069	2.078	2.086	2.095	2.103	2.112	2.120
164	2.008	2.017	2.026	2.035	2.043	2.052	2.061	2.070	2.078	2.087	2.095	2.104	2.112	2.121	2.129

165	2.017	2.026	2.035	2.044	2.052	2.061	2.070	2.079	2.087	2.096	2.105	2.113	2.122	2.130	2.138
166	2.026	2.035	2.044	2.053	2.061	2.070	2.079	2.088	2.096	2.105	2.114	2.123	2.131	2.139	2.148
167	2.034	2.044	2.053	2.062	2.070	2.079	2.088	2.097	2.105	2.114	2.123	2.132	2.140	2.148	2.157
168	2.043	2.053	2.062	2.071	2.079	2.088	2.097	2.106	2.114	2.123	2.132	2.141	2.149	2.158	2.166
169	2.052	2.062	2.071	2.080	2.088	2.097	2.106	2.115	2.124	2.132	2.141	2.150	2.159	2.167	2.176
170	2.061	2.070	2.079	2.088	2.097	2.106	2.115	2.124	2.133	2.141	2.150	2.159	2.168	2.176	2.185
171	2.070	2.079	2.088	2.098	2.106	2.115	2.124	2.133	2.142	2.151	2.160	2.169	2.177	2.186	2.194
172	2.079	2.088	2.097	2.106	2.115	2.124	2.133	2.142	2.151	2.160	2.169	2.178	2.187	2.195	2.204
173	2.087	2.097	2.106	2.115	2.124	2.133	2.142	2.151	2.160	2.169	2.178	2.187	2.196	2.205	2.214
174	2.096	2.106	2.115	2.124	2.133	2.142	2.151	2.160	2.169	2.178	2.187	2.196	2.205	2.214	2.223
175	2.105	2.114	2.124	2.133	2.142	2.151	2.160	2.169	2.178	2.187	2.196	2.205	2.214	2.223	2.232
176	2.113	2.123	2.133	2.142	2.151	2.160	2.169	2.178	2.187	2.196	2.205	2.214	2.223	2.232	2.241
177	2.122	2.132	2.142	2.151	2.160	2.169	2.178	2.187	2.196	2.205	2.215	2.224	2.233	2.241	2.250
178	2.131	2.141	2.151	2.160	2.169	2.178	2.187	2.197	2.206	2.215	2.224	2.233	2.242	2.251	2.260
179	2.140	2.150	2.159	2.169	2.178	2.187	2.196	2.206	2.215	2.224	2.233	2.242	2.251	2.260	2.269
180	2.148	2.158	2.167	2.177	2.186	2.195	2.205	2.214	2.223	2.232	2.242	2.251	2.260	2.269	2.278
181	2.157	2.167	2.176	2.186	2.195	2.204	2.214	2.223	2.232	2.241	2.251	2.260	2.269	2.278	2.287
182	2.166	2.176	2.185	2.195	2.204	2.213	2.223	2.232	2.241	2.250	2.260	2.269	2.278	2.287	2.296
183	2.174	2.184	2.193	2.203	2.212	2.222	2.231	2.241	2.250	2.259	2.269	2.278	2.287	2.296	2.305
184	2.183	2.193	2.202	2.212	2.221	2.231	2.240	2.250	2.259	2.268	2.278	2.287	2.296	2.305	2.314
185	2.191	2.201	2.211	2.221	2.230	2.240	2.249	2.259	2.268	2.277	2.287	2.296	2.305	2.314	2.323
186	2.200	2.210	2.220	2.229	2.239	2.249	2.258	2.268	2.277	2.286	2.296	2.305	2.314	2.323	2.332
187	2.208	2.218	2.228	2.238	2.247	2.257	2.267	2.277	2.286	2.295	2.305	2.314	2.323	2.332	2.341
188	2.217	2.227	2.237	2.247	2.256	2.266	2.276	2.286	2.295	2.304	2.314	2.323	2.332	2.341	2.351
189	2.226	2.236	2.246	2.256	2.265	2.275	2.285	2.295	2.304	2.313	2.323	2.332	2.341	2.350	2.360
190	2.234	2.244	2.254	2.264	2.274	2.283	2.293	2.303	2.312	2.322	2.331	2.341	2.350	2.359	2.369
191	2.242	2.253	2.263	2.272	2.282	2.292	2.302	2.312	2.321	2.330	2.340	2.350	2.359	2.368	2.378
192	2.251	2.261	2.271	2.281	2.291	2.301	2.311	2.321	2.330	2.339	2.349	2.359	2.368	2.377	2.387
193	2.260	2.270	2.280	2.290	2.300	2.310	2.320	2.330	2.339	2.348	2.358	2.368	2.377	2.386	2.396
194	2.268	2.279	2.289	2.299	2.308	2.318	2.328	2.338	2.347	2.357	2.367	2.377	2.386	2.395	2.405
195	2.277	2.287	2.297	2.307	2.317	2.327	2.337	2.347	2.356	2.366	2.376	2.386	2.395	2.404	2.414
196	2.285	2.296	2.306	2.316	2.326	2.336	2.346	2.356	2.365	2.375	2.385	2.394	2.404	2.413	2.423
197	2.294	2.304	2.314	2.324	2.334	2.344	2.354	2.364	2.374	2.383	2.393	2.403	2.413	2.422	2.432
198	2.302	2.313	2.323	2.333	2.343	2.353	2.363	2.373	2.382	2.392	2.402	2.412	2.422	2.431	2.441
199	2.310	2.321	2.331	2.341	2.351	2.361	2.371	2.381	2.391	2.401	2.411	2.421	2.431	2.440	2.450

all, theoretically, should be correct to four places when accuracy to three places is desirable. However, the importance of these theoretic considerations is diminished by the fact that weight and height can be measured accurately to but three significant figures.

SUMMARY

Values of the Du Bois formula for human surface area are tabulated by unit cm. and kg. intervals for individuals of 110 to 200 cm. in height and of 20 to 110 kg. in weight.

Thanks are due George E. Redfern for the greater part of the routine calculation.

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STABILITY OF STANDARD KAHN ANTIGEN*

By Pearl L. Kendrick, B.S., and Dora Jenks, B.S., Lansing, Mich.

A PROBLEM of practical importance in connection with a serologic test for syphilis is the stability of the antigen used. Early in 1924 the method of preparing antigen for the Kahn test was so standardized as to assure an unusual degree of uniformity.[1] It was still to be determined how long the antigen thus prepared would continue to react with the same degree of sensitiveness as when made; whether this sensitiveness would be affected by the temperature of storage, and whether an antigen would deteriorate after cholesterinization. In an attempt to determine these points the following experiments were carried out.

EXPERIMENTAL

Effect of storage at various temperatures.—Given amounts of Kahn standard antigen were sealed in glass ampules and stored at each of the following temperatures: 0°, 21°, 37°, and 54° C.† At the end of one day, three days, one, two, five, nine and nineteen weeks, respectively, each of the four experimental antigens was tested for its sensitiveness with 20 serums of varying potency—employing the Kahn routine test. After each period of storage, the antigens at the different temperatures gave results comparable among themselves and to a freshly prepared standard antigen used as a control. When the results did not show absolute agreement, the variations were not more than one-plus except in two cases where there was a difference of two-plus. Quite obviously, such small variations may be considered as insignificant. Table I gives the findings after five, nine and nineteen weeks' storage. The results recorded are those obtained in the second tube of the routine test (0.025 c.c. antigen dilution plus 0.15 c c. serum).

Effect of boiling.—Since the antigen was apparently unaffected at a temperature of 54° C, experiments were carried out to determine whether it was sufficiently stable to withstand boiling. Five sealed glass ampules of antigen were immersed in boiling water and removed at the end of one, five, fifteen, thirty and sixty minutes, respectively. These five antigens were then tested with serums of varying potency, using standard antigen, unheated, as a control and employing the second tube of the routine test (0.025 c.c. antigen dilution plus 0.15 c.c. serum). The results indicated that the variations in the reactions given by the antigens kept at the boiling temperature of water for various periods, as compared with unheated control antigen were so slight as to be practically insignificant. Table II gives the findings with 40 serums.

Results with antigens stored for a year.—In the first experiment, the findings (Table I) indicated that the results with standard antigen after a period of nineteen weeks' storage were practically the same as those given

*From the Bureau of Laboratories, Michigan Department of Health, Lansing, Mich.
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†The antigen stored at 0° C. contained a precipitate which redissolved when the ampule was placed in warm water for a few minutes.

by the control antigen which was freshly prepared. In order to observe the effect of storage for a still longer period, comparative tests with serums of varying potency were carried out, using a standard antigen kept at 21° C. for a year, and one prepared a week before its use in the tests. Three different old antigens were used—antigen A in series 1, antigen B in series 2 and antigen C in series 3. The results with each of the old antigens were comparable with those obtained with a freshly prepared antigen. The few slight variations were such as might be expected with two different antigens. Table III gives the findings with 170 serums. Each finding is the average of the results obtained in the three tubes of the routine test.

Age and Cholesterinization.—An alcoholic extract which had been stored a year was cholesterinized a day before its use and tested with serums in comparison with the same lot of alcoholic extract cholesterinized immediately after its preparation and a week before its use in the tests. The findings with both antigens, as well as with a freshly prepared antigen, were practically

TABLE I

RESULTS WITH ANTIGEN AFTER VARIOUS PERIODS OF STORAGE AT DIFFERENT TEMPERATURES

PERIOD OF STORAGE OF ANTIGEN	NUMBER OF SERUMS	STANDARD ANTIGEN CONTROL	STORAGE TEMPERATURES			
			0°C.	21°C.	37°C.	54°C.
5 weeks	3		++++	++++	++++	++++
	6		+++	+++	+++	+++
	1		++	++	+++	++
	4		++	++	++	+
	2		++	++	+	−
	1		+	+	+	+
	1		−	+	−	−
	2		−	−	−	−
9 weeks	4	++++	++++	++++	++++	++++
	3	++++	++++	++++	++++	++++
	2	++++	++++	++++	++++	++++
	2	++	++	++	++	+
	2	+	+	+	−	−
	7	−	−	−	−	−
19 weeks	1	++++	++++	+++	+++	+++
	4	+++	+++	+++	+++	+++
	1	+++	++++	+++	++	++
	2	+++	++	++	++	++
	3	++	++	++	++	++
	1	++	++	+	+	+
	2	+	+	+	+	+
	6	−	−	−	−	−

TABLE II

RESULTS WITH ANTIGEN AFTER VARYING PERIODS IN A BOILING WATER-BATH

NUMBER OF SERUMS	STANDARD ANTIGEN (CONTROL)	PERIODS OF BOILING				
		1 MIN.	5 MIN.	15 MIN.	30 MIN.	60 MIN.
9	++++	++++	++++	++++	++++	++++
2	++++	++++	+++	+++	+++	+++
4	+++	+++	+++	+++	+++	+++
1	++	++	++	++	++	++
1	++	+	++	+	+	+
1	++	+	+	+	+	+
1	+	+	+	++	+	++
2	+	+	+	+	+	+
19	−	−	−	−	−	−

TABLE III

RESULTS WITH ANTIGENS STORED FOR ONE YEAR

SERIES 1			SERIES 2			SERIES 3		
NO. OF SERUMS	NEW ANT. CONTROL	OLD ANT. A	NO. OF SERUMS	NEW ANT. CONTROL	OLD ANT. B	NO. OF SERUMS	NEW ANT. CONTROL	OLD ANT. C
19	++++	++++	14	++++	++++	19	++++	++++
5	++++	+++	2	++++	+++	3	+++	++++
1	++++	++	2	+++	++++	6	+++	+++
1	+++	+++	9	+++	+++	1	++	++++
2	+++	++	3	+++	++	3	++	+++
1	+++	+	1	++	+++	9	++	++
2	++	++	2	++	++	4	+	++
5	++	+	1	+	++	1	+	+
9	+	+	8	+	+	2	±	+
15	–	–	8	–	–	12	–	–

TABLE IV

RESULTS WITH NEWLY CHOLESTERINIZED ANTIGEN COMPARED WITH OLD CHOLESTERINIZED ANTIGEN

NO. OF SERUMS	DATE OF PREPARATION OF ALCOHOLIC EXTRACT		
	MARCH 20, 1925	MARCH 1, 1924	
	DATE OF CHOLESTERINIZATION		
	MARCH 21, 1925	MARCH 2, 1924	MARCH 21, 1925
11	++++	++++	++++
1	++++	++++	+++
1	++++	+++	++++
3	++++	+++	+++
2	+++	+++	+++
1	+++	++	++
2	++	++	++
2	++	+	+
2	+	+	+
15	–	–	–

Date of tests: March 27, 1925.

in agreement. Table IV shows the results with 40 serums. Each finding is the average of the results obtained in the three tubes of the routine test.

SUMMARY

Kahn standard antigen was found to be practically unaffected by storage at 0°, 21°, 37° and 54° C. up to a period of nineteen weeks. It withstood the temperature of boiling water for at least an hour. After storage at 21° C. for one year, three different antigens gave results comparable to those given by freshly prepared antigen. An antigen stored for a year after cholesterinization gave practically the same results as the alcoholic extract stored for the same length of time but cholesterinized just before its use.

CONCLUSION

Our findings indicate that Kahn antigen is a relatively stable product.

From a practical standpoint, the findings suggest that Kahn antigen can be stored safely at room temperature (in the dark) for long periods; it is unaffected by temperature changes accompanying distribution through the mails and frequent cholesterinization of the alcoholic extract is unnecessary.

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LABORATORY METHODS

AN OUTFIT FOR THE COLLECTION OF SERUM BY PHYSICIANS FOR THE DARK-FIELD EXAMINATION IN SUSPECTED SYPHILIS*

By William Royal Stokes, M.D., and C. LeRoy Ewing, Baltimore, Md.

THE importance of an early diagnosis of syphilis is so apparent that this fact hardly warrants any further comment, since it has been sufficiently emphasized by a large number of authors. Some of these authors point out that the Treponema pallidum can often be demonstrated in the secretions taken from any open lesion as well as from the primary lesion. It has also been detected in the secondary rash and from the inguinal glands. It is important, therefore, to make an early diagnosis from the primary lesion if possible, by a microscopic examination of the serum obtained from such a lesion; if this method is extensively practiced, a much larger percentage of permanent cures can be effected. It is often the unrecognized and neglected cases originating from an atypical papule, herpes, or other such lesion, which are the most stubborn, and which develop the chronic lesions of the circulation, abdominal viscera, or nervous system, which exact the high toll and final mortality of syphilis.

Newsholme[1] quotes Osler's statement that syphilis is more destructive to life than tuberculosis, cancer, or pneumonia, although this comparison is based on an estimate of the deaths from syphilis listed under such causes as deaths of the insane, heart disease, paralysis, cerebral hemorrhage, premature birth, cancer of the tongue, and other conditions, and do not appear in the vital statistics listed as syphilis deaths. Newsholme attempted to "get nearer the truth regarding syphilis as a cause of mortality," and his table on page 484 shows a death rate ascribable to syphilis of 119.5 per 100,000 of the population.

It is therefore important to make an early diagnosis in as many cases as possible. Without early diagnosis, we can scarcely hope to effect any large reduction in syphilis mortality. Our Health Department, in keeping with the general custom of other departments, maintains a number of clinics in which the dark-field examination is made. Many cases of syphilis are detected in the early stage of the disease at these clinics and the proper treatment instituted. In addition, the Bureau of Bacteriology makes a dark-field examination from the primary lesion of any patient sent directly to the laboratory with a note from his physician requesting such an examination. Comparatively few patients, however, come to the Bureau for such examinations, and the laboratory, therefore, has recently devised an outfit in which physicians

*From the Bureau of Bacteriology, Health Department, Baltimore, Md.
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can obtain dark-field specimens in their offices, the outfit being sent to the Health Department for examination. This method overcomes the objections which many patients have against appearing in person at the Health Department for examination, and it maintains the due privacy which a physician may wish to preserve for his patients.

Each outfit contains a fine pipette with a rubber nipple. The serum is obtained either by capillary attraction or by a slight pressure upon the nipple. After the collection of the specimen, the pipette is placed within a glass tube and is sent to the laboratory as soon as possible, as our experiments have demonstrated that the motility often becomes sluggish about two hours after collection. In a circular sent out in the monthly Health News, the physicians have been urgently requested to use this outfit. A physician can obtain it at any one of about 160 culture stations distributed throughout the city or at the Health Department.

If a negative report is received, the physicians are then urged to send their patient to the Health Department for another examination, and the view is expressed in the circular that "if the physicians will cooperate with the Department in detecting these early cases of syphilis, it is felt that many cases will be saved from those future developments which may eventually destroy the life of the patient. By carrying out this technic, it is hoped that the mortality from the organic lesions due to syphilis may be gradually reduced."

EXPERIMENTAL WORK

Schereschewsky[2] has performed some experiments which should be considered in connection with this outfit. He uses a capillary pipette with a rubber nipple to scrape away the superficial covering of the lesion, and then uses the pipette to place four drops of normal salt solution over the lesion, and mixes this salt solution with the serum that exudes from the surface. He then uses a U-shaped capillary tube open at both ends, with one side

TABLE I

TABLE SHOWING DURATION OF MOTILITY OF TREPONEMA PALLIDUM IN POSITIVE CASES

TYPE OF LESION	LENGTH OF TIME BETWEEN COLLECTION AND DARK-FIELD EXAMINATION	CHARACTER OF MOTILITY	TEMPERATURE OF INCUBATION
Chancre	5 hours	Wavy and side to side movement	85° F.
Chancre	5 "	Active, wavy, and side to side movement	80° F.
Chancre	2 "	Slight side to side movement	98° F.
Chancre	2 "	Slight side to side and wavy movement	68° F.
Chancre	5 "	Slight side to side and wavy movement	70° F.
Chancre	24 "	Motility absent	70° F.
Chancre	2 "	Slight side to side and wavy movement	70° F.
Chancre	2 "	Slight side to side and wavy movement	70° F.
Chancre	5 "	Motility absent	70° F.
Chancre	8 "	Active, wavy, and side to side movement	70° F.
Mucous Patch	4 "	Active, wavy, and side to side movement	80° F.

having a diameter three times greater than the other, the serum being drawn up into the finer capillary end by capillary attraction. This capillary tube is detached from the other tube and sealed at both ends, and can then be transported from a clinic to the laboratory for examination. He states that he has observed motility in the Treponema pallidum for three weeks, when preserved in such closed tubes. This method, therefore, might be easily used for sending specimens by mail.

Willett,[3] of the St. Louis Health Department, in extragenital lesions which are not readily accessible, has used a capillary pipette with a rubber nipple to advantage.

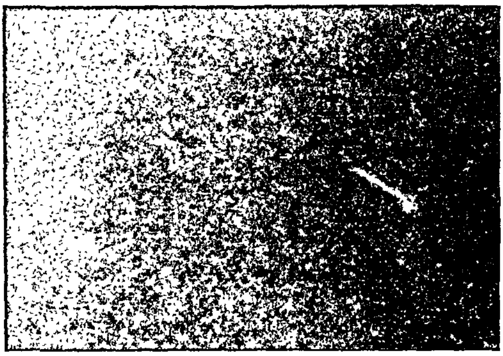

Fig. 1.—Shows two formalinized specimens of the Treponema pallidum. (Mr. Hermann Becker.)

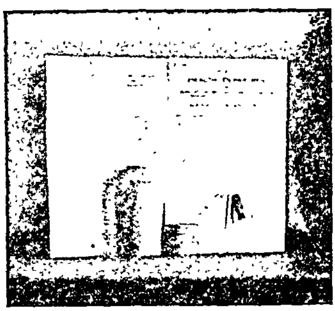

Fig. 2.—Photograph of the outfit for the collection of serum described in the text. (Mr. T. C. Buck.)

In obtaining specimens in the city, however, we have found the use of the second U-tube unnecessary, and have simply collected pure serum in the pipettes by the method which will be described below in detail.

These specimens have been collected from patients who reported to the Health Department or to some of our neighboring clinics. The table shows the time which intervened between the collection and the examination, including the variety or loss of motility after different periods of time.

The table shows that most of our specimens were active after five hours, but, as one was negative at the end of this time, we would suggest that no greater interval than four hours elapse between the collection of the specimen

and the dark-field examination. These specimens were all taken from cases which were demonstrated as positive immediately before the use of the tube.

APPLICATION TO TEACHING STUDENTS

We have also been able to use our method in demonstrating to students the appearance of the Treponema pallidum. By collecting a specimen at the clinic, we have been able to use it later in several dark-field apparatus and to demonstrate to medical students, at the University of Maryland, the appearance and activity of the organism. By mixing an equal quantity of a 10 per cent solution of formalin with several drops of the serum and spreading it out between a slide and cover slip, we have preserved such specimens for several months. This is accomplished by carefully sealing the sides of the cover slip with balsam in order to prevent evaporation. The student first becomes familiar with the appearance of the formalinized organism under the dark-field, and then later examines motile, active specimens obtained from the clinic. We consider these demonstrations a definite part of the crusade against the mortality from syphilis, since with more medical men familiar with the organism, a larger number of early diagnoses will be made.

Fig. 1 is a photomicrograph of the Treponema pallidum preserved for class instruction, which also shows the appearance of a spirochete which has been obtained from an active lesion and which has lost its motility. Even under this condition, we consider the appearance of such a specimen just as characteristic as that of a fixed spirochete which has been stained by any of the ordinary staining methods.

DESCRIPTION OF TECHNIC

The technic of collecting the specimen is described in the circular which accompanies all of our outfits. Fig. 2 shows a photograph of this outfit.

INSTRUCTIONS FOR OBTAINING A SPECIMEN FOR DARK-FIELD EXAMINATION FROM A PRIMARY LESION IN SUSPECTED SYPHILIS.

The specimen should be collected preferably with the patient lying on a couch. The lesion is thoroughly washed and sponged with warm tap water, using absorbent cotton. The physician should rub the lesion with a sterile gauze sponge until bleeding is produced, and this can only be aided by a moderate amount of pressure. It is important to remove the superficial epithelium in order to reach the deep portion of the lesion. The blood should be wiped away, and nothing further should be done until the lesion is bathed in serum. A curette may be used instead of gauze.

The operator then applies the open tip of the pipette to the serum, and sucks up enough serum by means of the nipple until the fluid reaches the even bore of the tube, as indicated by the red line. The serum should be as free from blood as possible.

The pipette should then be replaced very lightly in the small glass test tube and not forced in, and great care should be taken not to expel the serum from the capillary pipette into the test tube. The specimen should then be brought at once to the laboratory during laboratory hours, and delivered personally to one of the bacteriologists, and no specimens will be accepted after 4 o'clock on week days, after 11 o'clock on Saturdays, or on Sundays or holidays.

The operator should always wear rubber gloves, which should be sterilized after the specimen has been obtained.

(1) The results by the zinc method were invariably too low.
(2) The silver and the direct methods gave results that were accurate only when the concentration was 4 mg. or more per 100 c.c. of blood (using a standard equivalent to 4 mg. per 100 c.c.).
(3) Few of the estimations, by any method, were accurate when the concentration was 1 to 3 milligrams.

By changing the amounts of zinc chloride and sodium carbonate used, we were able to secure results by the zinc method that checked with those by the other two methods when the concentration was 4 mg.

We tried adding pure uric acid to the blood unknown just before adding the uric acid reagent and cyanide. When 5 c.c. of solution was added, containing the same amount of uric acid as the standard, the increase in volume spoiled the estimation (color unsatisfactory). We next reduced the amount of added uric acid to 0.5 c.c., using a more concentrated solution which contained five times as much uric acid as Folin's dilute standard. This addition was equivalent to an increase of 2 mg. per 100 c.c. of the original blood.

By such addition of uric acid to the unknown, we secured accurate results by all three methods, with solutions corresponding to blood specimens containing 1 to 6 mg. per 100 c.c.

Having modified three methods so that they checked accurately on pure uric acid solutions, we were ready to try them on normal blood samples. It was thought best to try each method with and without the addition of uric acid to the unknown making six estimations on each sample of blood. A summary of the accuracy of the results is given in Table I. It will be seen at a glance that by far the greatest accuracy is secured when uric acid is added to the unknown (our modification). Estimations by methods Nos. 2, 4, and 6 show this clearly. Over half of the estimations by the unmodified direct method (No. 5) were off color, making color comparison almost impossible. What causes this, we do not know. However, the addition of uric acid to the unknown (No. 6) certainly makes it easy to match the colors, so that the results are excellent. Similar good results were obtained by the modified precipitation methods (Nos. 2 and 4).

The third part of the experimental work was on blood from hospital

TABLE I

COMPARATIVE ACCURACY OF 6 URIC ACID METHODS ON 55 NORMAL BLOOD SPECIMENS

METHOD NUMBER	1	2	3	4	5	6
Number of estimations:						
Accurate	18	52	16	55	3	48
Excess	2	0	20	0	9	2
Slight excess	0	0	14	0	0	3
Slight deficiency	29	3	0	0	0	2
Off color, poor estimations	3	0	4	0	14	0
Total specimens	52	55	54	55	26*	55

Range of normals: 1.5 to 3.7 mg. uric acid per 100 c.c. blood.
Average of normals: 2.4 mg.
*This method was discontinued because the type of color made the estimation unsatisfactory.
Methods: Zinc method without added uric acid, No. 1, and with added uric acid, No. 2.
Silver method without added uric acid, No. 3, and with added uric acid No. 4.
Direct method without added uric acid, No. 5, and with added uric acid, No. 6.

TABLE II

COMPARISON OF RESULTS BY THREE MODIFIED METHODS (URIC ACID ADDED) ON HOSPITAL BLOOD SPECIMENS

METHOD	MODIFIED ZINC (NO. 2) MG.	MODIFIED SILVER (NO. 4) MG.	MODIFIED DIRECT (NO. 6) MG.	VARIATION NO. 2 FROM NO. 4 MG.	VARIATION NO. 6 FROM NO. 4 MG.
Case 1	0.8	0.9	0.8	0.1	0.1
2	1.1	1.1	1.0	0.0	0.1
3	1.7	1.6	1.7	0.1	0.1
4	2.0	2.1	2.0	0.1	0.1
5	2.0	2.1	2.0	0.1	0.1
6	2.0	2.1	1.9	0.1	0.2
7	2.4	2.5	2.5	0.1	0.0
8	2.5	2.6	2.6	0.1	0.0
9	2.6	2.6	2.6	0.0	0.0
10	3.0	3 0	3.1	0.0	0.1
11	3.5	3.6	3.6	0.1	0.0
12	3.5	3.6	4.0	0.1	0.4
13	3.5	3 7	3 7	0.2	0.0
14	3.7	3.7	3.7	0.0	0.0
15	4.1	4.0	4.2	0.1	0.2
16	4.1	4.4	4.4	0.3	0.0
17	4.2	4.2	4.4	0.0	0.2
18	5.7	5.7	6.0	0.0	0.3
19	10.0	10.3	10.2	0.3	0.1
20	10.2	10.2	10.2	0 0	0.0
21	10.6	10.7	10.7	0.1	0.0
22	11.4	11.4	11.2	0.0	0.2
23	13.0	13.1	13.1	0.1	0.0
24	13.1	13.2	13.3	0.1	0.1
25*	13.3	13.1	13.3	0.2	0.2
Average Variation				0.09	0.1

*Uric acid not added (methods 1,3 and 5 used).

cases. Because of the superiority of Methods 2, 4, and 6 as shown by the previous work on normals, we decided to use only these three methods. An attempt was made to select cases in which uric acid findings might be significant. The estimations on 25 cases and their variations are given in Table II. For purposes of comparison the estimation by the silver method was taken as standard, and the variation from it was computed. The results show that all three methods are quite satisfactory from the standpoint of accuracy.

TECHNIC

Direct Estimation Method (Modified Folin-Benedict).—Measure exactly 5 c.c. of blood filtrate and 0.5 c.c. of modified standard uric acid solution into a test tube. Into a second tube measure 1 c.c. modified standard uric acid and 4.5 c.c. of water. Now add to each of the tubes 3 drops of 20 per cent lithium sulphate, exactly 1 c.c. of uric acid reagent, and finally, just 2 c.c. of sodium cyanide solution. Mix and, after standing two minutes, heat in a boiling bath 70 to 80 seconds. Cool in water to room temperature, dilute to 25 c.c., and mix. Estimate at once setting the standard at 20 mm.

Calculation: $\left(\frac{20 \times 4}{\text{mm. unknown}}\right)$ minus 2 = mg. uric acid in 100 c.c. blood.

Zinc Chloride Check Method (modified Morris and Macleod).—To 5 c.c. of blood filtrate in a centrifuge tube add 2 c.c. of water, exactly 0.4 c.c. of 1 per cent zinc chloride solution, and 0.7 c.c. of 2 per cent sodium carbonate solution (made from anhydrous carbonate). Stir with a rod, then rinse the

rod with a few drops of water. Balance with another tube. After standing five minutes, centrifuge for three minutes. Pour off the clear liquid (not used). To the precipitate add 1 c.c. of Folin's HCl-NaCl (10 gm. sodium chloride dissolved in 100 c.c. of tenth-normal HCl) and stir with a rod. Dilute with 4 c.c. of water and stir until all is dissolved. Rinse the rod. Pour the liquid (drain fully) into Tube I. Also add 0.5 c.c. of modified standard uric acid solution. Into Tube II measure 4.5 c.c. of water and 1 c.c. of modified uric standard. Into each tube put three drops of 20 per cent lithium sulphate, 1 c.c. of uric reagent, and 2 c.c. of sodium cyanide. Mix, after two minutes heat in the bath for 80 seconds, cool, dilute to 25 c.c., and mix. Estimate with the colorimeter and calculate as in the direct method.

Reagents.—

(1) Folin's sodium cyanide solution: Dissolve 15 gm. of purest cyanide in 100 c.c. of tenth-normal NaOH. Impurities cause the fresh solution to give considerable blue color with the uric acid reagent, interfering with the estimation. After two weeks, the impurity gives little color. When four months old, it should be discarded or else purified by aeration to remove ammonia. Very old solutions give an improper blue color.

(2) Folin's standard uric acid solution. (a) Strong stock solution: dissolve 0.5 gm. of pure uric acid and 0.25 gm: of lithium carbonate in 75 c.c. of hot water (60°) in a 500 c.c. flask. When dissolved clear, cool and add 175 c.c. of water and 12.5 c.c. of 40 per cent formalin. Shake, add 3 c.c. of glacial acetic acid, mix, and fill to the mark. This solution keeps for a very long time. (b) Modified dilute standard: mix 2 c.c. of stock solution, 50 c.c. of water, 5 c.c. of two-thirds normal sulphuric acid and 0.5 c.c. of formalin in a 100 c.c. flask and fill to the mark. This is five times as strong as the dilute standard used by Folin. Make the solution every two months.

(3) Folin's original uric acid reagent: Dissolve 50 gm. of C. P. sodium tungstate in 350 c.c. of water; add 40 c.c. of 85 per cent phosphoric acid, and heat the flask, having a reflux tube attached. Boil for two hours, cool, and dilute to 500 c.c.

(4) One per cent zinc chloride solution. When first made the solution is likely to be cloudy, but it is perfectly satisfactory when filtered.

DISCUSSION

Although half of our hospital cases fail to show uric acid retention, all were selected on account of the possibility of increase of uric acid content of the blood. They included cases of nephritis (of various types), prostatic obstruction, arthritis, gout, hypertension, and leucemia. A complete blood chemistry examination was made in almost every case. The report of the results and the discussion of their relation to uric acid retention are reserved for a future paper.

We recommend the zinc method for use whenever it is necessary to make a check on the results by the direct method, since our modifications have made the method reliable. It is much cheaper and a little quicker than the silver lactate method.

Estimations by the silver method were reported by Rogers[6] to be inaccurate because of a rapid change due to exposure to light. Our work does

not confirm this. Our results have been practically identical when an estimation exposed to ordinary diffused daylight for fifteen minutes was compared with one run in the dark.

It is quite possible that an occasional clinical blood specimen will give too high an estimation by the direct method because of the presence of certain substances other than uric that give a blue color with the reagent. Lennox and O'Connor,[7] also Bulmer, Eagles, and Hunter[8] reported a number of blood samples showing large variations in results by the precipitation and the direct methods. Others have secured excess estimations on animal blood by the direct method. Of the 80 specimens examined by us only one (Case 12) showed a distinct variation (10 per cent).

Folin[9] has recently put out an improvement of his uric acid reagent. This has been freed of molybdenum (present as an impurity) and is supposed to give the blue color only with uric acid. It is to be hoped that this reagent will make the direct estimation reliable in the case of all blood specimens. In the series of estimations which we have made by the modified direct method (No. 6), using both the original and the improved reagent, the results were identical. However, when the unmodified direct method is used, the new reagent gives a better type of color than does the old reagent. The preparation of the improved reagent is rather tedious. We believe that in all but a very small percentage of cases, the old reagent is quite satisfactory if our modified technic is followed.

While it is true that the addition of uric acid to the unknown is not necessary for a uric acid content above 6 mg., yet, estimations made with added uric acid are satisfactory at all levels.

CONCLUSIONS

1. For estimating blood specimens that have a normal uric acid content, our suggestion of adding uric acid to the unknown must be followed to secure sufficient color and a proper type of color for accurate estimation.

2. The direct method, which has previously been very unsatisfactory, is now reliable in most cases, provided our technic of adding uric acid to the unknown is followed.

3. The zinc precipitation method as modified by us has proved a very satisfactory check method, and the cheapness of the reagents as compared with those of the silver method, make it desirable. It is not necessary to use this method in preference to the direct method unless uric acid retention is indicated by the latter.

4. The normal uric acid content of the blood in 55 individuals varied from 1.5 to 3.7 mg. per 100 c.c., and averaged 2.4 mg.

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A METHOD FOR STAINING NERVE CELLS EN BLOCK WITH BASIC ANILINE DYES*

By Frederic Proescher, M.D., and Albert S. Arkush, A.B., Agnew, California

THE original Nissl method[1] for the elective staining of nerve cells is, in spite of its technical difficulties, still the method of choice. It aims to produce a standard nerve cell picture, regardless of the true morphologic structure of the nerve cell, as long as the conditions under which it is obtained are identical. The structures made visible may, or may not, be identical with the living organism. Nevertheless, the method furnishes a standard of comparison for the living cell structures.

Nissl has shown that 96 per cent alcohol is the fixative par excellence. According to Nissl, sections should be made from the unembedded material and stained in an aqueous solution of a basic aniline dye (methylene blue) followed by differentiation in aniline alcohol, etc.

The main difficulty in this method is the sectioning of the unembedded material. Sections of the pia and degenerated areas, as well as of whole hemispheres, are especially difficult to obtain. The staining and differentiation require a great deal of patience and skill in order to obtain satisfactory specimens. In fact, the entire process is difficult and comparatively limited from a technical standpoint.

To overcome these difficulties, celloidin and paraffin embedding and staining with toluidin blue, thionin, or cresyl violet is advocated,[2] but none of these combinations give specimens favorably comparable to those from unembedded material.

A large number of staining experiments with alcohol-fixed brain material have shown us that certain basic aniline dyes, in alcoholic solution and at various temperatures and ion concentrations, easily penetrate brain and spinal tissue blocks to practically any depth.

We have tried a large number of basic aniline dyes in alcoholic solution and find that methylene azur (thiazin red) and toluidin blue are the only stains of value. Neutral red, indulin, pyronin, nigrosin, cresyl violet, methylene blue, and methyl green have been tried without success. As a stain, we recommend methylene azur[3] which can be easily prepared in any desired quantity from methylene blue by oxidation with sodium peroxide. The crude methylene azur should be recrystallized, at least three times, from redistilled alcohol. The dyes are dissolved in alkalized chemically pure ethyl or methyl alcohol.

Recent investigations have demonstrated the importance of the ion concentration of staining solutions.[4] We adjusted the alcoholic solution to ion concentrations varying from P_H values of 7.4 to 12.

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*From the Pathological Laboratory, Agnew State Hospital, Agnew, California.
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The blocks, after removal from the staining solution, were uniformly stained a deep blue. The cortex is always of a deeper shade than the medullary substance, and stains much more rapidly. Some of the stain is removed by a short washing of the blocks in absolute methyl or ethyl alcohol. Immersion in xylol, which follows, stains the xylol bluish violet to violet, indicating that the free base of the dye is removed. If the xylol is changed until colorless, the blocks are almost translucent and of a deep bluish violet. The subsequent immersion of the blocks in molten paraffin removes no stain.

Microscopic examination of the sections shows that the nerve cells and especially the basophilic substance, including the nuclei, are electively stained, while the background is almost completely unstained. Other nonnervous elements are stained.

Fig. 1.—Section of paretic brain showing ganglion cells with basophilic substances, glia cells, and vessel with perivascular infiltration. Operative 4 mm., ocular No. 2.

Satisfactory penetration may be obtained for small blocks at room temperature or at 37° C. We find, however, that at 58 to 60 degrees, blocks of 4 to 8 mm. are well penetrated in three to five hours. Periods two to five times as long are required at the lower temperatures, depending on the size of the blocks.

While the alcoholic solution easily penetrates unaided, we find that the addition of glycerin adds both to the penetration of the stain and to the mechanical facility of sectioning. A range of two per cent to five per cent of glycerin is offered, as the penetration desired will vary with the type of work. Below two per cent there is an inappreciable effect and above 5 per cent the sections come off the microtome too soft and the staining is too diffuse. It seems that there is a definite relation, however, between the optimum amount of glycerin and the P_H of the staining solution (possibly due to impurities in the glycerin). Increase in alkalinity lowers the increased penetration due to glycerin, so that the higher value given above is recommended with high alkalinity.

The P_H values of 7.4 to 12 were found to give well differentiated sections. Seven and six-tenths to 10 are probably optimum limits. Outside these values the staining is either too weak or too heavy.

The staining results indicate that the basophilic substance of the nerve cells and certain nonnervous cellular elements can be electively stained, if xylol is used as the differentiating and clearing medium.

The question of whether or not the staining process is of a chemical or physicochemical nature, we leave undecided. Probably the basic elements of the nerve cells and other nonnervous elements form an insoluble dye salt with the stain, while the unstained nervous tissue has no affinity for the dye or forms a loose combination from which the stain is easily extracted.

A block has recently been stained by us which shows the protoplasmic and fibrous glia, as well as the basophilic substances of the nerve cells, stained and differentiated. This represents an almost ideal formula, and efforts are being made to determine how the result may be uniformly obtained. This particular block was stained for eleven and one-half hours at room temperature in a solution containing a small amount of eosin and a trace of toluidin blue, as well as methylene azur. It forms an excellently combined picture of the protoplasmic and fibrous glia and that of Nissl, for ganglion cells.

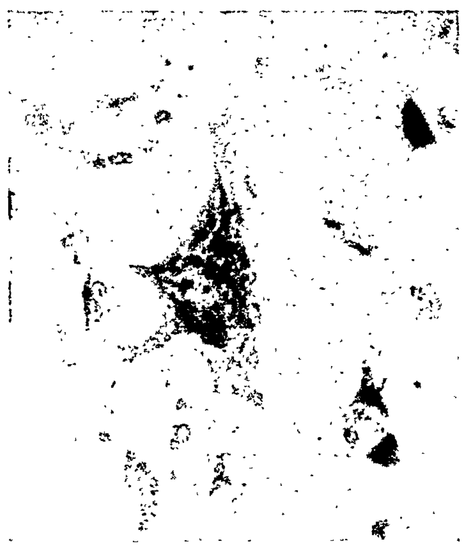

Fig. 2.—Ganglion cell shown in picture. 1. Oil immersion 1/12, ocular No. 2.

METHOD OF PROCEDURE

For preparing the staining solution, chemically pure methyl or ethyl alcohol is brought to the desired hydrogen-ion concentration by adding the sodium (or potassium) hydroxide as described. It will be found that 0.25 c.c. to 2.00 c.c. of the alcoholic alkaline solution approximately covers the useful P_H range. This may be compared with a colored buffer standard, if desired, but once determined for the character of work to be performed, such comparison will be found no longer necessary. Small changes, such as those introduced on addition of the dye (carefully prepared), are inconsequential.

1. To each 100 c.c. of the alkalized methyl or ethyl alcohol add 0.25 gm. of thiazin red.

2. Immerse the thoroughly fixed brain tissue (fixed in 96 per cent alcohol for at least five days) in the staining solution. This should be at least

two to three times the volume of the block. Keep here for five hours at a temperature of 56 to 60 degrees

3. Remove the stained blocks and wash in methyl or ethyl alcohol. If glycerin is added to the staining solution, the alcohol should be renewed two or three times in ten to fifteen minutes to remove as much of the glycerin as possible and facilitate the penetration of xylol.

4. Immerse in xylol for three to four hours, changing the xylol at least three times.

5. Pass the block through xylol paraffin for several hours and then in molten paraffin for one to three hours

6. Cut the block into thicknesses of 4 to 6 microns.

7. Mount the sections and dry them for several hours at 37° C. Glycerin egg-albumen should not be used in mounting if it is desired to keep the

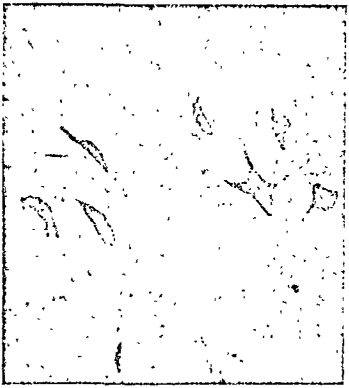

Fig. 3.—Motoric ganglion cells from the anterior horn of a normal spinal cord. Objective 1 mm, ocular No. 2

sections. In case it is used, care should be taken that the sections are thoroughly dried.

8. Melt the paraffin carefully over the flame, remove the paraffin with neutral xylol, and mount in xylol-colophonium.

With the above described staining method, an elective staining of the basophilic substances of nerve cells, including nuclei and nuclear membrane, glia cell bodies, and mesenchymal cellular elements of the vessel walls is possible. The neurofibrillae remain unstained, the fibrous glia either colorless or of a pale blue stain.

Compared with sections stained with the standard Nissl method, it seems that the protoplasmic structures, in general, are stained somewhat more deeply. The pigments are more distinct and certain nuclear elements, not shown by the original method, are stained metachromatically. This new factor can be added to the standard picture established by Nissl.

The following additional advantages of this method may be noted:

a) The cumbersomeness of the Nissl method is avoided.

b) Much thinner sections can be obtained and the finer details of the histologic changes of the nerve cells are more easily observed, and without precipitation of the dye.

c) Complete serial sections can be made.

d) Sections made by this method have been kept for six months without appreciable change. With the Nissl method, the majority of the sections deteriorate in short time.

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A METHOD OF BLOOD GROUPING WHERE ONLY ONE KNOWN GROUP IS AVAILABLE*

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THE increasing frequency of blood transfusion has necessitated the development of simple laboratory methods for determining the suitability of donors, and has made blood grouping a procedure which the laboratory worker is frequently called upon to do. I have found the method herein outlined to be of greater convenience than the emergency practice of testing for crossagglutination when standard agglutinating sera of Groups II and III are not available. On the staff of every hospital or laboratory there is almost certain to be, at least, one individual known to possess a Group II blood. By the following technic we have found it possible to not only determine the suitability of a donor, but to group both donor and recipient.

TECHNIC

Secure 3 c.c. of blood from a Group II individual, from the prospective donor, and from the recipient. Allow one-half of each blood specimen to fall into three separate centrifuge tubes containing 1 per cent of sodium citrate in physiologic salt solution. Throw down the citrated cells, remove supernatant fluid with a pipette and wash the sediment with physiologic salt solution several times, repeating the procedure with the citrated half of each of the three blood specimens. In each instance, make a 10 per cent emulsion of the red cells in physiologic salt solution.

Remove the serum from the coagulated remainder of each of the three blood specimens, and clear by centrifuging.

*From the Clinical Laboratory, Riverside, California.
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Carry out the following procedure with the known Group II serum and cells against the recipient's serum and cells and against the prospective donor's serum and cells:

Into a small test tube (7 mm. by 10 cm.) pipette 0.2 c.c. of the Group II serum and 0.2 c.c. of the unknown cells and into a similar tube, pipette 0.2 c.c. of the unknown serum and 0.2 c.c. of the Group II cells. Mix the contents of each tube thoroughly by shaking and place in a water-bath at 37° C. for two hours. At the end of incubation, examine for agglutination.

INTERPRETATION OF RESULTS

If no agglutination occurs in either tube, the unknown belongs to Group II.

If agglutination occurs in both tubes, the unknown belongs to Group III.

If agglutination occurs only in the tube containing the unknown serum and the Group II cells, the unknown falls in Group IV.

If agglutination occurs only in the tube containing the Group II serum and the unknown cells, the unknown belongs to Group I.

TABLE I

	TUBE 1 (GROUP II SERUM AND UNKNOWN CELLS)	TUBE 2 (UNKNOWN SERUM AND GROUP II CELLS)
Group I	Agglutination	No Agglutination
Group II	No Agglutination	No Agglutination
Group III	Agglutination	Agglutination
Group IV	No Agglutination	Agglutination

While in our laboratory we prefer the macroscopic or tube method, using substantial quantities of serum and cells, the same method may be employed with much smaller quantities of blood secured from the puncture of finger or ear, the mixtures being made as hanging drop preparations on a concave microscope slide with two concavities, and examined after thirty minutes under the 16 mm. objective.

SUMMARY

A method of blood grouping is described requiring only such glassware and equipment as the clinician has available in his office for the microscopic examination of urinary sediment; where but a single blood specimen of a known group is at hand. Needless to say a similar technic may be evolved for use with a known Group III. It is suggested that the physician who is removed from laboratory facilities, have his own or the blood of the office assistant grouped, so that a test similar to the one outlined may be performed before emergency transfusion. In grouping blood specimens of the physician and office assistant in ten offices of general practitioners, one or more Group II or III persons were found in all but one office. Where laboratory service is at hand, we draw an additional 2 c.c. of blood from prospective donors for the routine Wassermann.

The principle of this technic was suggested to me by G. R. Rea, Professor of Bacteriology, University of Saskatchewan, Canada, and is gratefully acknowledged.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

Leveriza, J. C.: Anti-Allergic Treatment of Bronchial Asthma. Jour. Philippine Med. Assn., June, 1925.

The author advances the suggestion that the poor results often obtained in the treatment of bronchial asthma with autogenous vaccines from the sputum may be due to the absence in the vaccine of bacterial proteins and bacterial exogenous products to which the patient may be sensitized.

In order to complete the vaccine and to secure the complete bacterial flora of the respiratory tract together with a concentrate of the bacterial exogenous products, he prepares a vaccine reinforced with the sputum filtrate.

The sputum is first examined for the presence of B. tuberculosis and, if this organism is absent, cultures are made on Loeffler's blood serum without preliminary plating or attempts to isolate specific strains.

An equal volume of 0.9 per cent saline is then added to the sputum, the mixture thoroughly shaken and incubated with the sputum culture for twenty-four hours at 38° C. The sputum-saline mixture should be shaken occasionally. After twenty-four hours incubation the culture is examined for spore-bearers. If these are absent, a suspension is prepared and sterilized by heating for one hour at 60° C. as usual.

The saline-sputum mixture is then centrifuged and the supernatant fluid filtered through a Chamberland or Berkefeld filter and tested for sterility.

The sterile filtrate is used to dilute the vaccine suspension and 0.5 per cent phenol is added as a preservative.

The initial dose is small and increased at two-day intervals until a mild general or focal reaction occurs, this dose then being continued until a reaction fails to occur when the dose is again increased cautiously. The maximum dose (1 c.c.) is repeated at regular intervals for at least ten injections.

The method is admitted to be empirical and inexact but better results are claimed in true bronchial asthma than have been secured with the usual autogenous vaccines.

Fildes, P.: Isolation, Morphology, and Cultural Reactions of B. Tetani. Brit. Jour. Exper. Path., April, 1925, vi, 62.

The author reports a study of B. tetani conducted upon a large number of pure cultures obtained by the method described.

The usual laboratory media were used without the addition of glucose. To gelatin and Loeffler's medium 0.5 per cent of blood laked with an equal volume of distilled water was added.

Slanted solid media was not used until after the upper edge of the slant showed slight signs of drying, in order to bring out the characteristic edge of the film-like growth described below.

When the organisms were few in number a preliminary enrichment culture was made in laked-blood broth and incubated in air for from two to four days, the longer period being preferred.

From this enrichment culture two drops were inoculated into the water of condensation of a solid slant. The tubes were then incubated under anaerobic conditions secured by the use of the electrically-heated jar described by Fildes and McIntosh.

After twenty-four hours at 38° C. a spreading growth is seen on the surface of the slant, different bacteria showing "high-water" marks indicating the limits of growth.

B. tetani presents this ability to spread up the surface of the slant more markedly than other anerobes. When it is present, there will be seen above the highest watermark on the

apparently unaffected surface of the medium a fine, almost structureless film at times detected only by a hand lens. When the uppermost edge of the film encroaches on the drying edge of the medium there may be seen a fine tangle of tenuous filaments. If smears from this film show the presence of tetanus bacilli, a transplant made from the upper edge of the film to the water of condensation of a new slant generally gives a pure culture, a third tube being rarely required.

The only organism resisting this method of isolation is B. proteus which can be removed by heating the material prior to inoculation.

Studies were made of 75 strains thus isolated, with interesting observations on the morphology and cultural characteristics of the organisms.

The article is illustrated with 30 figures.

Boquet, A. and Negre, L.: Methylic Antigen in the Study of Tuberculous Antibodies and in the Treatment of Experimental Tuberculosis in Smaller Laboratory Animals. Presse Med., Paris, Oct. 3, 1925, lxxix, 1315.

The authors, from Calmette's laboratory, describe their attempts to prepare a "stable, unalterable, specific antigen" sufficiently sensitive to reveal all the fixing bodies in a tuberculous subject.

The method of preparation of the antigen is as follows:

Human and bovine tubercle bacilli are grown in glycerin broth for six weeks. The cultures are sterilized by exposure to 120° C. for thirty minutes, filtered through paper, and the bacterial residue mixed. The bacterial mass is then washed on the filter with distilled water and then dried in a vacuum or in the incubator at 38° C.

It is then transferred to a flask, and for each centigram 1 c.c. of acetone is added, the mixture being allowed to stand for forty-eight hours with occasional shaking. The acetone is decanted, the bacilli rapidly dried and emulsified in absolute methyl alcohol using a volume equal to the amount of acetone used. This mixture is placed in the incubator at 38° C. for ten days, being shaken three to four times daily. It is then filtered, the filtrate constituting the antigen.

For complement-fixation tests the antigen is diluted with normal saline 1:20.

Before dilution, to dissolve the precipitate which forms on standing, the flask is placed in water at 45 to 50° C. for two or three minutes. The dilution is then made in a clean, dry flask, the saline being added at first drop by drop and later more rapidly.

The resultant emulsion should be opalescent and decidedly opaque.

The reactions with this antigen are said to have a high degree of specificity. Cross fixation with syphilitic serum, however, is said to occur.

The extract in concentrated form was also used for the treatment of experimental tuberculosis in rabbits and guinea pigs and for the production of complement fixing in rabbits.

For this purpose the extract is diluted with an equal volume of distilled water, added at first drop by drop, then more rapidly with constant agitation. The milky emulsion thus obtained is placed on a water-bath at 48-50° C. until all the alcohol is evaporated. One centimeter of the remaining emulsion is equal to 1 centigram of dried bacteria or 5 centigrams of fresh, washed bacteria. For the production of complement-fixing bodies 2 to 3 c.c. are injected into rabbits intravenously at intervals of once or twice a week.

For treatment 1 c.c. amounts are injected intraperitoneally into guinea pigs and subcutaneously into rabbits once or twice a week.

Encouraging results are claimed.

Cunningham, R. S., Sabin, F. R., Sugiyama, S., and Kindwall, J. A.: The Rôle of the Monocyte in Tuberculosis. Bull. Johns Hopkins Hosp., October, 1925, xxxvii, No. 4, 231.

This paper, which is in the nature of a preliminary though extensive report, presents the results of a prolonged study of experimental tuberculosis in the rabbit culminating in the concept that tuberculosis is a disease affecting primarily one single strain of cells, namely, the monocytes.

Evidence is presented to show that in this disease there is a monocytosis (reticular cells, monocytes, and their derivatives, epithelioid and giant cells); that the tubercle bacillus becomes a parasite in the monocytes, and that the predominence of these cells in the lesions is correlated with an increase of monocytes in the circulating blood.

A new avenue of experimental attack is thus uncovered and a new concept of immunity in this disease advanced.

The studies, cytologic in character, were made by a "supravital technic."

Slides and cover-glasses are thoroughly cleaned by immersion for three to four days in concentrated sulphuric acid to which is added a crystal of potassium bichromate; washed in running hot water, then in distilled water, and kept in 80 per cent alcohol. Before use, the alcohol is removed with cheesecloth and the slide thoroughly flamed.

Any trace of grease is fatal to the method.

Saturated alcoholic solutions of vital neutral red and Janus green are diluted for use as follows:

Neutral red: 20 to 30 drops to 10 c.c. of absolute alcohol. The color should be rose-red.

Janus green: 3 drops to 1 c.c. of absolute alcohol is suitable for studies of the blood; for tissue cells, the dye concentration is increased to not more than 6 drops.

The cooled slide is flooded with the mixed dyes thus prepared, the stain being immediately poured back into the bottle. It may be used over and over until contaminated by dust or grease. It must not touch the fingers when poured on the slide or be allowed to remain long enough for the alcohol to evaporate. The slides are dried in an upright position.

Blood is collected on a cover-glass which is inverted on the prepared slide. The preparation is then rimmed with vaseline of high melting point and examined on a warm stage at 38° C.

Lung, liver, and kidneys are gently scraped and mounted as a blood film. The film must be thin. Lymph glands, spleen, and bone marrow preparations are secured with capillary pipettes. Cells from subcutaneous tissues are best secured by producing an artificial edema through the injection of neutral red 1: 10,000 in Ringer's solution and mounting small bits of the resulting gelatinous tissue.

By the study of such preparations the authors believe that they have demonstrated the rôle of the tubercle bacillus in transforming monocytes into epithelioid and giant cells, the pictures seen by them being minutely described and well illustrated.

From their studies the authors conclude that progress in studies of tuberculosis will depend upon attempts to ascertain the mechanism underlying the overproduction of monocytes in tuberculosis and their transformation into epithelioid cells; that the monocyte is the cell primarily affected and which serves as a host to the invading organisms; and that the discovery of the substances affecting them will be the first step in the evolution of a specific immunity.

"With a substance by which the overproduction of monocytes might be checked, then the problem of immunity in tuberculosis might be more analogous to the problem in those diseases which have already been controlled."

Castellani, A.: Biochemical Characteristics of Certain Bacteria When Living in Association or Artificially Mixed and When Living Alone. Brit. Med. Jour., Oct. 24, 1925, No. 3382, 734.

This observation reports some interesting and not readily explainable effects of symbiosis or artificial mixed growths of bacteria upon the biochemical reactions of the organisms.

The B. typhosus, for example, produces acid but no gas when grown in maltose broth. Morgan's bacillus produces neither acid nor gas on the same medium. When B. typhosus and Morgan's bacillus are grown together on maltose broth the expectation would be that only acid would be produced, whereas, as a matter of fact, both acid and gas production occur. The same reaction occurs with mixtures of B. typhosus and B. proteus and with other organisms in mixed cultures as well. It is thus evident that a mixture of two species differing in their biochemical characteristics, when grown together, may produce biochemical reactions common to neither organism. Castellani comments upon the obscurity surrounding this reaction and the necessity for its further study.

Baer, J. L., and Reis, R. A.: The Sedimentation Test in Gynecology. Amer. Jour. Obst. and Gynec., September, 1925, x, No. 3, 397.

The authors report the results of 192 observations on 100 cases. They recommend on the score of simplicity and accuracy, the Linzenmeier-Friedlander technic which in their hands was performed as follows:

Hard glass tubes containing more than 1 c.c. are prepared. The tubes are 6.5 cm. in length and 5 mm. in diameter. Marks are made at the 1 c.c. level and 6, 12, 18, and 24 mm. below this point.

Into a dry Luer syringe is placed 0.2 c.c. of freshly prepared 5 per cent sodium citrate solution and 0.8 c.c. of blood is drawn. The syringe is shaken to mix the blood and citrate and the mixture placed in the tube. The time is taken when the blood is tubed. The tubes are allowed to stand at room temperature and readings are made and the time noted when the line of demarcation has reached the 18 mm. level.

They emphasize the necessity for a uniform technic in order that the results of different observers shall be made comparable. They report their results in the graph below:

Min.	
205	
195	
180	Normal Women
165	Sactosalpinx
150	Uncomplicated Myomata
135	Salpingitis isthmica Nodosa
120	Ovarian Cyst
105	Salpingitis Chronica
90	
75	
60	
45	Salpingitis Subacuta
30	Salpingitis Acuta
15	
5	
0	

Cummer, L., and Lyne, F. R.: The Wassermann Reaction—A System Using an Excess of Amboceptor. Amer. Jour. Syph., Oct., 1925, ix, No. 4, 765.

The authors report their experience with a Wassermann technic devised by Ecker in which the influence of natural antisheep amboceptor in the tested serum is rendered innocuous through the use of an excess of amboceptor in the hemolytic system.

The basic principles of the method involve: (1) The use of an excess quantity of hemolytic amboceptor. (2) The accurate titration of complement in the presence of excess amboceptor. (3) The employment as antigen of a normal heart extract prepared according to the method of Ecker.

Method: Glycerinated antisheep amboceptor is titrated in a range of dilutions of 1:1000 to 1:10,000 or higher; 0.5 c.c. of these dilutions is placed in a series of tubes and 0.25 c.c. of 5 per cent sheep cell suspension, 0.25 c.c. of 1·10 complement added and finally 0.5 c.c. normal saline to make a final volume of 1.5 c.c.

The complement is the pooled serum of several pigs. The tested serum is inactivated at 56° C. for twenty-five minutes.

The antigen is prepared by extracting fresh powdered heart muscle with boiling acetone under a reflux condenser for four hours, cooling in the ice box, filtering, and extracting for the same period in the same apparatus with absolute methyl alcohol, acetone-free, and evaporating to a volume three times the weight of the dried powder.

The cell suspension is sensitized as follows: The amboceptor is diluted so that 1000

units are contained in 50 c.c. of normal saline and this amount added slowly with rotation to an equal amount of 5 per cent cell suspension. After thorough mixing the flask containing the mixture is placed in a 38° C. water-bath for thirty minutes. After this period the flask may be kept at room temperature or in the ice box. In each tube of the complement titration and in the tests, 0.5 c.c. of this sensitized cell suspension containing 5 units of amboceptor is used.

For the complement titer the following amounts of 1:30 complement are placed in a series of 7 tubes: *0.09, 0.12, 0.18, 0.21, 0.24, 0.30,* and *0.33* c.c. In each tube place 0.5 c.c. of sensitized cells except the last tube in which 0.25 c.c. of nonsensitized cells is placed, this being the control tube.

Make the total volume 1.5 c.c. with saline and incubate 30 minutes at 38° C. The tube containing the least amount of complement showing absolutely complete hemolysis is the unit. In the reactions 1:10 complement is used and 2 units added to each tube.

The reactions are thus set up: Three tubes, the third being the serum control, are used for each serum. Serum is added as follows: 0.1 c.c., 0.05 c.c., and 0.1 c.c. To the first tube is added 0.1 c.c. of antigen dilution. After fifteen minutes at room temperature, 2 units of 1:10 complement are added to all tubes. Shake and incubate in the ice box for sixteen to eighteen hours (overnight). Then add saline to make a volume of 1 c.c. and 0.5 c.c. of sensitized cell suspension added. Mix and incubate at 38° C. in the water-bath for thirty minutes. Readings are made after a short period to permit partial settling.

For spinal fluids three tubes with corresponding controls are used containing 0.1 c.c., 0.4 c.c., and 0.7 c.c.

The method is stated to be delicate and not to give nonspecific reactions and but few anticomplementary reactions.

Muller, L.: An Apparatus for Anaerobic Cultures. Compt. rend. Soc. de biol. Paris, July, 11, 1925, xciii, 436.

The apparatus is based upon the absorption of oxygen by a heated catalyst. An electric current is not required. The method is designed especially for tube cultures.

A large test tube, 30 cm. × 25 mm. is fitted with a rubber stopper having a central and a lateral perforation.

Through the lateral opening is passed a copper or brass tube 3 to 4 mm. in diameter; through the central opening is passed a copper rod having a diameter of 7 to 8 mm. This rod is about 18 cm. in length and projects into the tube about 6 cm. The external portion is curved at a fairly acute angle. The internal portion is surrounded throughout its length by a fine copper or brass netting which holds a small amount of palladium-coated asbestos. To avoid danger of explosion, this first layer of wire netting is covered by a second of the same material held apart by loosely wound copper wire except at the ends of the cylinder thus formed, where it is tightly fastened by wire ligatures.

The tube should be of such a size that when the apparatus is adjusted there should still be a space about 22 cm. long to receive the culture tube.

In the bottom of the tube is placed a small tube about 2 cm. in diameter half filled with dextrose gelatin containing methylene-blue in the proportion of 1:2000. This serves as an indicator of the degree of anaerobiosis. On top of this is placed a small piece of crumpled filter paper. The indicator tube is placed in the tube first and then the culture tube.

When the culture has been inserted the rubber stopper, moistened with glycerin, is fitted in place, a three-way stopcock is attached to the copper tube by a small piece of rubber tubing and a partial vacuum produced by an air pump. Hydrogen gas is then passed into the apparatus. To start catalysis the curved end of the copper rod is passed through the Bunsen flame several times or inserted into boiling water. After twenty minutes, during which the formation of vapor shows the fixation of hydrogen, the rubber tubing is closed by a pinchcock or plugged with a piece of glass rod and the apparatus incubated.

The complete absorption of oxygen is shown by the decolorization of the indicator (methylene-blue-gelatin).

Howard, H. J.: The Rôle of the Epithelial Cell in Conjunctival and Corneal Infections. Jour. Ophthalmol., December, 1924, vii, 909.

Howard, writing from Peking, in this paper describes his experience with the methods devised by Lindner for the study of eye infections. The gonococcus, pneumococcus, Koch-Week's bacillus, diphtheria bacillus, and influenza bacillus by means of these studies are seen to be parasites of the epithelial cells, proliferating on the surface of the cells and drawing nutriment from them. As they penetrate the deeper layers, they provoke proliferation of the cells and other evidences of reaction. The older epithelial cells covered with a turf of bacteria are cast off; and the younger cells become phagocytes which digest the bacteria.

The methods follow:

The specimen is procured, after anesthetizing the eye with an instillation of 5 per cent cocaine followed by an instillation of 10 per cent cocaine, by gently scraping the palpebral conjunctiva with a platinum spatula.

Dry Fixation: Scrapings are transferred to cover-glasses, allowed to dry in the air, and fixed by flooding with absolute alcohol. The alcohol is allowed to dry spontaneously. The cover-glass is then placed film side down in the staining solution, either Giemsa's stain or Lindner's contrast stain. Giemsa's stain is used in the proportion of 5 to 10 drops to 10 c.c. of distilled water.

Lindner's contrast stain: 10 c.c. distilled water, 5 to 10 drops of Giemsa's stain, 1 drop concentrated alcoholic solution of methylene-blue, and 1 drop of 1 per cent acetic acid solution. Wipe away the metallic scum and mix. Stain for one-half to one hour. Dry and mount in cedar oil—balsam must be avoided.

Wet Fixation: The cover glass is immediately placed in the solutions indicated. A small tube or jar with rounded bottom so that the film does not come into contact with the container may be used. (1) Ten c.c. concentrated sublimate solution, 20 c.c. 95 per cent alcohol. Thirty minutes. (2) Thirty per cent alcohol two hours. (3) Fifty per cent alcohol two hours. (4) Seventy per cent alcohol, twelve to twenty-four hours. (5) Eighty per cent alcohol to which a few drops of Lugol's solution have been added, twelve to twenty-four hours. The iodine is to remove the mercury. (6) Ninety-five per cent alcohol, twelve to twenty-four hours. (7) Giemsa or Lindner stain. (8) Wash quickly with absolute alcohol. (9) Wash quickly with xylol. (10) Shake off excess of xylol, mount in cedar oil and examine.

Detailed directions are also given for the preparation of sections and the pictures seen are minutely described and well illustrated.

Austin, J. H., and Cullen, G. E.: Hydrogen Ion Concentration of the Blood in Health and Disease. Medicine, August, 1925, iv, 275.

This paper presents a "convenient outline for those whose interests are clinical rather than physiological."

From their own work and the literature the authors summarize the present knowledge concerning the P_H of the blood in health and disease.

Under normal conditions the reaction of the blood is stabilized through the excretion of nonvolatile acids and bases through the kidney, change in the base binding properties of the hemoglobin with oxygenation and reduction, and the excretion of carbon dioxide through the lungs, the last two processes being the more rapid and important.

By the term "P_H of blood" is meant the P_H of the blood serum. Normally this is from 7.3 to 7.4. Colorimetric determination is the method of choice for clinical investigation in the authors' opinion.

Extracellular fluids (edema, nonpurulent joint effusions, etc.) have the same P_H as the serum. Purulent fluids are more acid than serum, ranging from 6.4 to 6.8.

In renal disease marked variations are encountered as might be expected, especially in association with uremia with which varying degrees of acidosis are associated.

In cardiac disease uncomplicated by renal impairment no alteration of moment in the P_H was found. Hyperpnea cannot be explained as a manifestation of acidosis in these cases.

Insulin stupor tends to be associated with an alkalosis and a high P_H. The acidosis of diabetes is, of course, associated with marked changes in P_H. Cases are reported in the literature evidencing recovery from reduction of the P_H to as low as 6.8 to 7.0.

In rheumatic fever there is no evidence of disturbance of the acid-base equilibrium, the plasma and joint fluid P_H being normal.

Dehydration in infants is associated with acidosis.

Fasting for fifty hours produces a depression of 0.02 in the P_H; after seventy-two hours a depression of 0.1 was encountered.

Anoxemia is associated with acidosis especially when due to capillary poisons. Marked fall in P_H occurs in anaphylaxis. A fall occurs in shock when the blood pressure is lowered by shock or hemorrhage; the importance of acidosis as a cause of shock remains to be shown. Changes in the blood P_H (fall) are seen in tetany, but their exact relationship to the disease is not established.

Hemorrhage first causes a drop in the P_H followed by a rise above normal.

In gastric diseases an alkalosis may result from constant loss of HCl and this may also occur after the Sippy treatment for ulcer.

In neoplasms (carcinoma), no significant changes in P_H occur.

Radiation seems to cause a temporary slight (0.02 to 0.09) increase in blood P_H.

In surgical anesthesia there is a combination of acidosis and depressed ventilation. There is, therefore, a constant drop in P_H associated with all forms of surgical anesthesia but satisfactory evidence if its clinical importance is lacking.

In lobar pneumonia no significant changes in the P_H were noted.

In various infections associated with severe febrile reactions a marked drop has been noted in the P_H of the serum.

In pregnancy a slight increase in the P_H has been recorded, probably as a secondary effect of hyperventilation rather than as due to a true alkalosis.

Douglas, S. R., and Meanwell, L. J.: A New Method for the Concentration of Bacilli in Tuberculous Milk. Brit. Exper. Path., Oct., 1925, vi, No. 5, 203.

By the usual methods of examining milk for the presence of tubercle bacilli many of the organisms are carried up with the fat globules and imbedded in the cream layer, while those which remain in the sediment are often in clumps and may thus be missed.

Douglas and Meanwell suggest a method in which these sources of error may largely be avoided.

Ten centimeters of milk to be examined are placed in a centrifuge tube fitted with a rubber-capped screw top. Five-tenths centimeter of liquor trypsin are added and the tubes incubated for three hours at 56° C. or six hours at 38° C. After cooling the tubes are opened, 5 c.c. of ether added, the cap screwed firmly in place, and the tubes shaken thoroughly at least 200 times and subsequently centrifuged for twenty minutes at 4000 r.p.m. After centrifuging three layers will be seen: ether-dissolved fat at the surface, a clear fluid below, and, between the two, a gelatinous disc which contains all the acid-fast bacteria.

A loopful of this gelatinous material is transferred to a slide, a drop of water added and a smear made. After drying at room temperature, the slides are placed into equal parts of alcohol and ether at room temperature for two hours and then stained by the usual Ziehl-Neelsen method. The method has successfully demonstrated tubercle bacilli when present in very small numbers and when the usual methods of examination had failed.

A modification of this method was devised for application to watery fluids such as urine, cerebrospinal fluid, pleural fluids, and the pus of cold abscesses which proved equally satisfactory.

Sputum or pus should first be treated with 20 per cent antiformin for one hour at 38° C. The other specimens are at once subjected to the procedures outlined below.

To 10 c.c. of the fluid to be examined, add 1 to 2 c.c. of oil (olive, cottonseed, or even liquid paraffin). Shake the mixture in order to emulsify the oil as thoroughly as possible. For specimens which have been treated with antiformin, liquid paraffin is more suitable than a vegetable oil.

The emulsion is allowed to stand for ten or fifteen minutes and 5 c.c. of ether is added. After closing the tube with the screw cap, again agitate thoroughly and centrifuge. After centrifuging three layers will be seen: an upper layer consisting of fats dissolved in ether, and a lower layer of watery fluid and debris, the middle layer containing the bacilli.

This intermediate layer varies in thickness, depending upon the character of the fluid in which the organisms were originally suspended. In the case of watery fluids, such as spinal fluid, it may be so thin as to be nearly invisible.

Smears are made and treated as above described for milk.

Where large volumes of fluid may contain only a few bacilli, such as in urine, oil is added to make about 5 per cent of the total volume and the mixture shaken until thoroughly emülsified.

The emulsion is then placed in a separating funnel and allowed to stand until the oil globules have risen to the surface. It is then drawn off and the oily layer run into centrifuge tubes and ether added. After again shaking, the tubes are centrifuged. In this case the oil-ether zone will be found much larger than the watery zone. Films are prepared and stained as above described.

The methods described have been very successful in the hands of the authors.

Osborne, E. D.: Microchemical Studies of Arsenic in Arsenical Pigmentation and Keratoses. Arch. Dermat. and Syph., December, 1925, xii, No. 6, 773.

This paper presents a method for the microchemical study of arsenic in relation to tissue cells and is a variation of that described by Brunauer. It may well be added to the armamentarium of the medicolegal worker.

The technic is as follows: (1) Fixation in 10 per cent formalin (4 per cent formaldehyde), twenty-four to forty-eight hours. (2) Wash in running tap water at lease six hours. (3) Cut tissue in small pieces not more than 2 mm. thick. (4) Place the pieces in a one ounce, ground glass stoppered bottle and nearly fill with fresh, neutral hydrogen sulphide. The stopper is greased with a small amount of petrolatum and tightly fastened in place with tape or cord. (5) Incubate at 70-80° C. for four days, or for six days at 56° C. The latter period better preserves the staining qualities of the tissue. (6) Wash the tissue, which will appear dirty gray to black, in running water six to twelve hours. (7) Dehydrate through 50, 70, 80, 95 per cent and finally absolute alcohol, each containing about 10 per cent of ether. (8) Imbed in celloidin and cut sections not over 5 microns thick. (9) Place the cut sections in 10 per cent HCl (diluted with alcohol), for twenty minutes. This procedure is essential in removing all sulphides except arsenic trisulphide, but it impairs the staining quality of the tissue. (10) Wash in 70 per cent alcohol for twenty minutes and stain in hematoxylin and eosin. (11) Differentiate in 95 per cent alcohol. (12) Clear with oil of cloves, removing the excess with xylol. (13) Mount in balsam. Arsenic trisulphide appears in tissue as nearly round or oval but somewhat irregular greenish-yellow crystals. When in focus the crystals appear solid with a bright halo; too near the objective, they have a heavy rim, a clear center, and a slight halo, beyond the focus they have a clear center, a faint rim and no halo.

Walzer, M., and Kramer, S. D.: Studies in Specific Hypersensitiveness. XVIII: An Indirect Method for Testing for Conditions of Atopic Hypersensitiveness (Preliminary Report). Jour. Immunol., September, 1925, x, No. 5, 835.

Scratch or intradermal tests for sensitization are sometimes confronted with practical difficulties as when, in infants, the cooperation of the patient is lacking or dermographia or other skin lesions interfere with the readings.

The method described is suggested as an alternative method in these types of cases. It is based upon the observation of Prausnitz and Klausner in 1921 that local sensitivity may be passively transferred by injecting into the skin of a normal individual the serum of a sensitized individual and later making skin tests on the normal person thus passively locally sensitized.

Method: When possible, blood is obtained from the presumably sensitized individual by venipuncture. About 4 c.c. of blood is discharged into a centrifuge tube containing sufficient 20 per cent sodium citrate to make a 1 per cent citrated mixture. After centrifugation the serum is pipetted off. Puncture of the heel or toe may be employed, aspirating the blood into a Wright capsule, and the serum obtained by centrifugation.

Where the passive agent is the parent, sterilization of the serum was considered unnecessary; where other individuals were employed, the serum was filtered through a Berkefeld filter.

If only a small amount of fluid was available the serum was diluted with an equal part of normal saline. It is important that the passive agent be free from a history of sensitiveness.

Local sensitization is accomplished by the intradermal injection of $\frac{1}{15}$ c.c. of the serum. About 30 sites 4 cm. apart were used, the anterior and external aspects of the arm and the flexor surface of the forearm being considered the most suitable. The wheals resulting from the intradermal injections are outlined with ink or indelible pencil. While sensitization is complete shortly after the introduction of the serum, the tests are better postponed to the following day.

The actual tests are made by the intradermic injection of about 0.01 c.c. of the antigen solution. Control tests are made at the same time with the same amount on the adjacent normal skin and the substances tested marked on a diagram in order to avoid confusion.

Reactions may be read in five to fifteen minutes. Positive reactions are the same as with the direct method. Erythema around a test in a sensitized site and absent in the control is significant and the test should be repeated on another site. Each site may be tested three to five times provided the same atopen is not used on the same site. Scratch method tests may be used but are not as clear cut as intradermic tests. A general reaction is to be avoided if possible. When it occurs all sensitized sites, tested or untested, may exhibit a marked localized urticaria though the subject is free from symptoms elsewhere. Though sensitivity persists for a month, tests are better expedited, four sittings on alternate days usually sufficing for the testing of a large number of substances.

The method is feasible but now requires study to determine the extent of its practicability.

Mulsow, J.: Culture Mediums for the Gonococcus. Jour. Infect. Dis., April, 1925, xxxvi, 419.

One pound of lean beef and 500 c.c. of water are mixed and allowed to stand forty-eight hours at 38° C. To the expressed juice add peptone 20 gm., potassium nitrate 2 gm., and an equal volume of 0.2 per cent agar melted and cooled to 60° C. Adjust the reaction to plus 0.9 to phenolphthalein. Autoclave fifteen minutes at 15 pounds, determine and adjust reaction if necessary. Best results are obtained when no adjustment of the reaction is required. A P_H of between 6.6 and 7.0 is suitable, 6.8 being preferred.

In this medium the gonococcus will grow as well if not better than contaminating organisms, the growth being confined to the surface.

If smears from the primary culture in this medium show organisms resembling the gonococcus, transplants are made to the differential plating medium described below.

One pound of lean beef and 500 c.c. of distilled water are allowed to stand in the ice chest overnight. To the expressed juice add 10 gm. peptone and 500 c.c. of melted 3 per cent agar cooled to 60° C. Adjust the reaction to plus 0.9 to phenolphthalein. Before the agar has cooled sufficiently to harden, autoclave twenty-five minutes at 15 pounds. Filter through moistened cotton and Canton flannel, place in 100 c.c. flasks and sterilize in the autoclave before the agar has solidified.

When ready to use add to the 100 c.c. flask 0.5 gm. of levulose or maltose and 1 c.c. of 0.4 per cent solution of cresol purple. Melt the agar, cool to 60° C. and add to 50 c.c. of ascitic fluid and pour plates.

Gonococcus colonies are easily distinguished from acid-forming organisms on this medium.

BOOK REVIEWS

(Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.)

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*Disease of the Male Organs of Generation**

A CONCISE exposition of diseases of the male generative organs, both functional and organic There appears an excellent discussion of sterility in the male.

Common Infections of the Female Urethra and Cervix†

THE major portion deals with the venereal infections, but other bacterial diseases of the localities under consideration receive due attention. The authors' discussion of methods of study should be of value to all who presume to undertake pelvic examination. The chapter on prophylaxis is complete and deals with both male and female prophylactic measures. There is a chapter on bacteriology by G. T Western and one on ophthalmia neonatorum by M. S. Mayou.

The Treatment of Fractures in General Practice††

FOR some time to come the general practitioner will still be called upon to treat fractures. Unfortunately for him, with the development of more satisfactory methods and with the application of the x-ray to this work, the public has come to expect and demand much greater skill and is more prone to hold the physician accountable for unsatisfactory results. It was with a realization of these two facts that the author offered this book to the practitioner. The first portion deals with general principles of diagnosis and treatment and discussion of complications, etc, while the remainder presents in detail the practical treatment of specific fractures. It is well illustrated and clearly and concisely written. Methods have not been simplified, as indeed they cannot be. The reviewer gains the impression that while the book is written for the general physician, it were far better were the treatment of fractures delegated more and more to the trained specialists. It is evidently not the intention of the author to create this impression, but

*Disease of the Male Organs of Generation. By Kenneth M. Walker, F.R.C.S., M.A., M.B., B.C. Cloth. Pp. 231. Price $4.00. Oxford University Press, Henry Frowde & Hodder & Stoughton, London. First printed 1923.

†Common Infections of the Female Urethra and Cervix. By Frank Kidd, M. A., M.Ch., (Cantab.), F.R.C.S. (England) and A. Malcolm Simpson, B.A., M.B., D P H. (Cantab.) with additional chapters by George T. Western, M D, and M. S. Mayou, F.R.C.S. Cloth. Pp. 191. Price $2.50. Oxford University Press. First printed 1921.

††The Treatment of Fractures in General Practice. By C. Max Page, D S.O., M.S. (Lond.), F.R.C.S. Cloth. Pp. 239. Price $4.00 Oxford University Press, Henry Frowde & Hodder & Stoughton, London First edition 1923.

we feel it an impression well worth acquiring. Nevertheless, the problem still exists for the man in general practice and he will welcome this handy reference volume.

*Gynecology with Obstetrics**

A COMPREHENSIVE textbook on obstetrics and general gynecology. Profusely and well illustrated. Little attention is given to the technic of operation—this with the exception of obstetrical conditions. In a prologue, the author gives an interesting historical sketch of obstetrics and gynecology from earliest times. This is followed by anatomic, embryologic and physiologic considerations. The work is primarily a textbook.

Modern Aspects of Syphilis†

MR. HAWKINS presents, in unusually readable form, a concise recapitulation of the problem of syphilis, both diagnostic and therapeutic, as it is interpreted at The Finger Clinic, in Vienna. The viewpoint is exclusively continental and should therefore be of comparative interest to Americans. The author emphasizes the importance of diagnostic lumbar puncture in every case of syphilis. He points out that in the salvarsan treatment of primary syphilis with negative blood Wassermann, a Wassermann must be performed after every injection. If but one time it becomes positive the case then falls into the category of secondary syphilis and requires more prolonged and intensive treatment. Thus, Wassermanns at the beginning and at the ending of the treatment of primary syphilis might be negative while one during the treatment is positive. Such a case would be erroneously diagnosed primary syphilis.

The value of mirion in preventing central nervous manifestations is discussed. The employment of nonspecific measures such as the injection of milk and inoculation with malaria plasmodia, particularly when used in conjunction with specific treatment, is discussed in detail. Intrathecal treatment is evidently not used at the Finger Clinic.

This volume should be highly recommended even to those who treat only an occasional case of syphilis, since it emphasizes the dangers of insufficient early treatment—quite a universal failing among the general practitioners—and presents an excellent routine for comprehensive treatment.

*Gynecology with Obstetrics. By John S. Fairbairn, M.A., B.M., B.Ch., (Oxon), F.R.C.P. (Eng.). Cloth. Pp. 769. Price $8.00. Oxford University Press. First printed 1924.

†Modern Aspects of Syphilis. By M. J. Morgan, B.A., M.B., B.Ch., B.A.O., N.U.I. Cloth. P. 136. Price $1.75. Oxford University Press, Henry Frowde & Hodder & Stoughton, London. First published 1923.

Books Received

DIE HEILUNG DER TUBERKULOSE und ihrer muschinfektionen (Skrofulose, Rheumatismus, Basedow-krankheit, U. A). Durch Kutanimpfung von Sanitatsrat Dr. Med. Wilhelm Ponndorf Vorstand der Thuringischen Impfanstalt in Weimar Zweite, Vermehrte und Verbesserte Auflage mit 1 kurve und 1 farbigen tafel. 1923 Verlag von F. C. W. Vogel in Leipzig.

KURZES LEHRBUCH der CHEMIE in Natur und Wirtschaft. Von Prof. Carl Oppenheimer, Dr. phil. et. med. Berlin nebst einer Einfuhrung in die Allgemeine Chemie von Prof. Johann Matula in Wien. 1923. Georg Thieme Verlag Leipzig.

HANDBUCH DER BIOLOGISCHEN ARBEITSMETHODEN. Unter Mitarbeit von 500 bedeutenden Fachmannern von Geh. Med.-Rat Prof. Dr. Emil Abderhalden Direktor des Physiologischen Institutes der Universität Halle a. d. Saale.

Abt. II. Physikalische Methoden, Heft 2 A. Kohler-Jena: Das Mikroskop und seine Anwendung. 1923. Urban & Schwarzenberg.

Abt. III. Angewandte chemische und physikalische Methoden, Teil 7, Heft 5. Pharmokologie, Pharmazie, Toxikologie. W. Storm van Leeuwen-Leiden: Physiologische Wertbestimmung von Giften und Giftkombinationen an Warmblutern und deren Organen. Hermann von Tappeiner-Munchen: Methoden beim Arbeiten mit sensibilisierenden fluoreszierenden Stoffen. Hermann Pfeiffer-Graz: Der Nachweiz photodynamischer Wirkungen flureszierender Stoffe am labenden Warmbluter.

Abt. IV. Angewandte chemische und physikalische Methoden, Teil 10, Heft 4. W. Klein und Marie Steuber-Berlin: Die Methodik des Gaswechselsan grossen Tieren, mit 8 Abbildungen. Paul Hari-Budapest. Elekerische Kompensationscalorimetrie, mit 12 Abbildungen. Otto Meyerhof-Berlin-Dahlem: Mikrocalorimetrie mit 14 Abbildungen. J. W. Capstick-Cambridge: Ein Calorimeter für das Arbeiten mit grossen Tieren. Mit 5 Abbildungen. 1925.

Abt. IV. Angewandte chemische und physikalische Methoden, Teil 10, Heft 3. Francis G. Benedict-Boston: Methoden zur Bestimmung des Gaswechsels bei Tieren und Menschen. Mit 74 Abbildungen. 1924.

Abt. V. Methoden zum Studium der Funktionem der einzelnen Organe des tierschen Organismus, Teil 5 A. Heft 2. Methoden der, usket-und Nervenphysiologie. R. Grammel-Stuttgart: Theoretische Grundlagen der Gelebkmechanik. E. Hirt-Munchen: Graphische Methoden zur Darstellung Normaler und Pathologischer Willkuikicher Bewegungsablaufe. Mit 59 Abbildungen im Text. Urban und Schwarzenberg. 1924.

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EDITORIALS

Milk Fever

A DEPANCREATIZED female dog, kept alive by daily injections of insulin and feeding with raw pancreas and sugar, became pregnant and gave birth to five pups. At various intervals during the pregnancy careful observations were made (by W. W. Simpson) of the carbohydrate balance, without finding that any change occurred. As has been pointed out in a previous editorial, this result does not support the view held by some, that the growing fetus can secrete sufficient insulin into the maternal organism to prevent the full development of diabetic symptoms when the pancreas is removed. But a fact of still greater significance observed in this animal, was that marked symptoms of hypoglycemia developed on the day *following* parturition, the cause for which was obviously the removal of glucose from the maternal organism to furnish the lactose of the milk. When this observation was made (January 2, 1925) we failed to realize that it gave the clue to the cause of milk fever in cattle, a fact which has been clearly established through the work of Widmark and Carlens.[1] In the first paper describing their work,

[1]Biochem. Ztschr., 1924, clvi, 453; ibid., clviii, 3, 81.

these authors show that the blood sugar of normal cows while not giving milk is 0.085 per cent, but that it falls to a level very near that at which hypoglycemic symptoms supervene (0.040 per cent) when the animals are being used for milking. A parallelism could also be demonstrated between the height of the blood sugar and the amount of milk which each animal yielded. Subsequently they found that the administration of insulin to normal cows caused the blood sugar to fall to below 0.040 per cent, when symptoms identical with those observed in milk fever supervened; namely, a gradually developing paralysis which ultimately became so severe that the animal collapsed, and although for some time after falling it attempted to raise its head, this finally ceased and the animal lay limp and helpless on its side.

The treatment for this condition obviously demands restoration of the blood sugar and it is significant that this is what occurs when the orthodox veterinary practice of blowing air into the udder is followed. Widmark and Carlens found, in confirmation of earlier work by Paul Bert and Porchet, that this operation causes a prompt rise in blood sugar in lactating animals, most pronounced in those giving the richest yields of milk and of only slight degree in poor milkers. This operation causes mechanical interference with the activity of the secretory cells of the gland, so that they fail to take up glucose from the blood. During the active secretion there is no doubt that amounts of glucose are being carried to the glands which are in excess of those present when no lactation is occurring; the sudden interference with the functioning of the cells then causes glucose to accumulate in the blood until a sufficient concentration is present to act as an inhibitory stimulus to the glycogenolytic mechanism, and so shut down the supply of blood sugar. That such a regulatory mechanism does come into play is indicated by the fact that the hyperglycemia following air injection is only temporary, the normal level of blood sugar being regained in about two hours.

—*J. J. R. M.*

Investigations on the Serodiagnosis of Syphilis

IN 1922, at the Pasteur Institute in Paris, the Health Committee of the League of Nations conducted a six day comparative investigation of the merits of various flocculation reactions in syphilis and the results of the Wassermann test.

Taking part in the investigation were serologists from Austria, Belgium, Denmark, Germany, Great Britain, and Poland.

So divergent were the results obtained that a second investigation and conference was held in Copenhagen, in 1923. Twenty-two workers participated, representing laboratories of London, Warsaw, Copenhagen, Westphalia, Paris, Berlin, Brussels, Heidelberg, and Florence. The United States was represented by two "observers" of the U.S.P.H.S.

The report of this conference is of some interest, first, because it represents a comparatively international effort to approach the specified problems; second, because the flocculation tests were compared, in most instances, by

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The next annual Convention of the American Society of Clinical Pathologists will be held in Dallas, Texas, April 15, 16, 17, 1926.

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CLINICAL AND EXPERIMENTAL

SOME RELATIONS BETWEEN THE CONCENTRATION OF BLOOD CORPUSCLES IN VENOUS AND CAPILLARY BLOOD AND THE BLOOD PRESSURE OF DIABETIC PATIENTS*

By Howard F. Root, M.D., J. W. Thompson, M.D., and R. R. White, M.D., Boston, Mass.

THE value of observation of the variations in the relative concentration of corpuscles in capillary and venous blood of diabetic subjects was suggested to us by the frequency of vascular disease and its sequelae in diabetics, and by the new point of view regarding the function of the capillaries brought out by Krogh[1] and his associates. Brown[2] has recently shown that in Raynaud's disease disturbed capillary tone, abnormal flow, or complete stasis may exist. The distribution of the blood corpuscles also has practical importance in relation to certain methods for determining blood volume, to capillary stasis such as occurs in traumatic shock, and possibly to the oxygen exchange in diabetes.

Methods.—Twenty-seven diabetic patients in the clinic of the New England Deaconess hospital on the service of Dr E P. Joslin including eleven men ranging in age from forty-six years to sixty-eight years and sixteen women ranging in age from twenty-eight years to seventy years, willingly served as subjects. In this group were two patients who had gangrene and suffered an amputation of the leg. Other patients were selected as having hypertension, hypotension, normal blood pressure, or acidosis. Counts of red and white corpuscles in venous blood and capillary blood of the same arm were made at 5:00 P.M. and at 7:30 A.M., just before the evening and morning meals. These hours were selected in order to eliminate, so far as possible, the effect of meals on the cell counts. The blood specimens were obtained at the same time or

*From the Diabetic Clinic of the New England Deaconess Hospital, Boston, Mass.
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within a few minutes of each other. In withdrawing the venous blood, a tourniquet was applied only long enough to inject the needle into the vein; when the tourniquet was removed and the needle allowed to remain quiet within the vein for ten or fifteen seconds. Six c.c. of blood were withdrawn and a count was made from the last drop drawn. The blood was taken in clean, dry syringes and a red and white count made immediately. The capillary blood was drawn by pricking the tip of the finger with a three-sided needle without compressing the finger.

Hayem's solution was used for making the red count and a freshly prepared 1 per cent solution of acetic acid for the white count. All counts were made by us using the same pipettes and the same two counting chambers. The counting chambers were made by Bausch and Lomb with a Neubauer ruling. Pipettes and counting chambers were standardized by the Bureau of Standards and further checked by comparing counts from the same drop of blood. Samples of such check counts are given below.

	Pipette No. 2840		Pipette No. 3830	
(1)	157	153	154	155
	165	164	160	161
	159	144	156	154
	148	163	150	157
	Count 5,016,000 (R. R. W.)		Count 4,992,000 (H. F. R.)	
(2)	162	181	175	153
	160	164	173	165
	180	174	182	160
	177	162	157	186
	Count 5,440,000		Count 5,404,000	
(3)	164	167	158	152
	152	156	153	146
	153	150	154	164
	160	148	168	164
	Count 5,000,000		Count 5,036,000	

In taking the capillary blood, precautions were taken to have the tip of the finger dry. Because the pain and discomfort is less with venipuncture, it was customary to take the venous blood first. The time elapsing between venipuncture and the collection of the capillary blood was not more than three minutes, usually less.

Because of the possible influence of pain involved in drawing blood, blood pressure readings were taken before and after from the opposite arm. In some instances blood pressure readings were made on the arm from which blood counts were taken. Since the same technic was used in the morning and evening with special care to allow an interval between the taking of the blood pressure and the drawing of blood, these results are considered comparable with the others. We used a Baumanometer with a visible mercury column. The same instrument was used for all determinations. In taking the readings, the systolic pressure was that at which the sound of the pulse beat could first be heard as the pressure was released from the cuff. The diastolic pressure was taken at that point where the intensity of the sound first began to diminish and not at the point where it disappears. Three separate readings were taken in every instance and the lowest of the three taken as the proper reading.

TABLE I

RED AND WHITE CORPUSCLES IN CAPILLARY AND VENOUS BLOOD OF 23 DIABETICS

CASE NO.	SEX	AGE YEARS	DURATION OF DIABETES YEARS	EVENING						MORNING						ARTERIO-SCLEROSIS
				Blood Pressure Mm. Hg.		Venous		Capillary		Blood Pressure Mm. Hg.		Venous		Capillary		
						R. B. C.	W. B. C	R. B. C.	W. B. C.			R. B. C.	W. B. C.	R. B. C.	W. B. C.	
2946	M	46.2	1.8	87	58	3.7	6.7	5.0	6.8	77	53	3.8	5.5	4.3	4.0	0
1947	F	56.4	3.0	108	58	5.3	5.4	4.4	6.1	110	68	4.7	5.9	4.3	5.4	0
3032	F	49.6	6.1	118	60	4.9	8.4	4.5	9.9	112	60	5.2	7.5	4.9	8.1	0
3767	F	70.9	2.4	132	65	5.6	8.4	5.9	10.7	150	65	5.7		4.9	7.6	0
3689	M	47.6	1.8	100	63	4.3	5.2	4.3	5.8	103	67	4.4	3.4	4.4	3.4	0
3034	F	40.8	1.9	82	70	3.9	4.6	3.8	4.8	76	56	4.6	4.5	5.0	3.0	0
3195	M	68.7	12.9	130	70	4.7	7.5	4.8	8.2	126	71	3.4		5.3		++
2876	F	63.7	5.4	129	70	4.8	4.7	4.4	4.5	131	70	4.2	4.8	4.1	5.0	+
3210	F	64.3	0.6	170	75	5.2	10.1	4.0	5.8	165	70	4.8	10.5	4.8	12.8	+++
3194	F	50.3	1.0	114	74	4.5	5.4	5.6	5.8	108	80	4.8	4.2	4.8	5.7	0
3067	F	40.9	1.6	116	80	5.0	9.8	4.4	10.1	102	70	5.2	6.2	4.7	9.4	0
3660	F	28.8	0.6	120	78	5.6	7.2	4.6	10.3	110	68	3.5		5.4	7.0	0
3192	F	62.2	8.8	184	92	4.6	4.5	5.5	6.3	148	79	4.9	5.9	4.8	7.4	+
3193	F	55.0	8.0	152	81	5.3	4.9	4.7	6.0	121	73	5.2	5.6	5.2	5.2	+
2818	M	66.8	6.3	130	82	4.7	4.3	4.2	5.2	122	90	4.2	4.6	4.6	4.6	+
3898	M	67.0	0.3	173	81	5.3	7.5	6.2	8.9	130	70	5.0	8.0	5.4	6.4	+++
3728	F	39.6	1.2	153	95	5.3	7.3	5.9	6.4	148	91	5.3	5.7	6.0	5.4	0
3169	M	68.3	10.9	150	90	5.4	8.6	5.0	8.0	148	85	4.7	6.1	4.1	7.8	+
3944	F	57.8	2.1	213	92	6.0	9.3	5.9	9.8	194	90	6.4	8.2	5.1	8.7	+++
3728	F	39.6	1.2	113	92	5.5	7.1	5.3	6.0	107	74	5.3	5.8	5.7	4.8	0
2980	F	62.8	7.0	190	100	4.6	8.2	5.8	8.2	120	92	5.2	4.4	4.8	3.7	+++
2980	F	62.9	7.0	236	106	4.1	5.8	4.8	6.7	128	56	5.6	6.0	4.8	6.9	+++
2937	F	48.6	0.8	182	110	5.5	10.9	6.9	10.5	174	110	6.6	8.1	6.1	6.2	0
1730	M	54.4	6.8	160	120	6.0		7.0		130	90	5.1	5.6	6.0	3.9	++

NOTE: Red counts are expressed in millions and white counts in thousands.

DISCUSSION OF RESULTS

1. We would first direct attention to the fact that all counts were made from blood from the arm. Had capillary blood from a toe been used there is little doubt that greater variations might have been found. This would have involved the factor of posture to a much greater degree, and we decided against making this comparison. In Table I is given a list of the patients by case number showing age, duration of diabetes, their maximum blood pressure morning and evening, and the corpuscle counts. This series is arranged in the order of their diastolic pressures. There does not seem to be any obvious relationship between the duration of the diabetes and the diastolic pressure. The case with the shortest known duration had rather a high diastolic pressure. Similarly age did not seem to be a dominant feature in determining the diastolic pressure.

Arteriosclerosis was evident in the peripheral arteries of eleven subjects in varying degrees designated by +, ++, +++. Case No. 2980 showed advanced vascular change in the eyegrounds. Case No. 3210, at autopsy one year later, was found to have calcification in the walls of the coronaries, aorta, and big vessels.

Our first consideration is the change in cell counts between night and morning. In the interpretation of cell counts, it is of importance to know what variation may be expected in counts made at different periods during the day. In Table II are shown the average differences in counts made just before supper and just before breakfast.

Table II

Average Differences in Cell Counts Without Regard to Sign Between Night and Morning

Red Corpuscles		White Corpuscles	
Venous	Capillary	Venous	Capillary
544,000	592,000	1,455	1,888

If the counts are averaged, the plus and minus differences balancing each other, such striking variations disappear. The average red count in venous blood for the entire group of patients in the evening was 5,001,500 and in the capillary blood 5,110,000. In the morning the average red count was 4,950,000 in venous and 4,974,000 in capillary blood. The white counts in the evening showed practically no variations, being 6,813 in the venous and 7,187 in the capillary blood. In the morning the white cells numbered 5,701 in the venous blood, and 6.097 in the capillary blood. The blood pressure on the other hand showed a considerable fall. The average systolic pressure in the evening for the entire group was 145 whereas in the morning it was 125. The diastolic pressure averaged 86 in the evening and 76 in the morning. Thus, with a considerable change in blood pressures, there was a very slight change in red cell counts but a definite decrease in the white count.

In Table III are shown the average variations in cell counts compared with the variation in blood pressure. It is seen that on the average the variations in blood pressures do not correlate with any characteristic changes

TABLE III

VARIATIONS IN NUMBER OF RED CORPUSCLES COMPARED WITH DIURNAL CHANGES IN BLOOD PRESSURE

Rise in systolic	7	3	4,889,000	4,863,000	4,743,000	4,477,000
Fall in systolic	24	19	4,987,000	5,153,000	4,985,000	5,128,000
Rise in diastolic	7	4	5,066,000	4,711,000	4,999,000	4,710,000
Fall in diastolic	14	14	4,899,000	5,144,000	4,810,000	5,146,000

Cases where no changes in blood pressure occurred are omitted

in the red cell count. The variations in the counts between morning and evening are on the average not large enough to be significant. However, if the patients are classified according to maximal diastolic pressures, then a very different picture is presented. Patients whose maximal diastolic pressure was 90 mm. of mercury or more are eight in number The average systolic pressure was 195 and diastolic 104 The average capillary count in the evening was 5,865,000 and the venous red cells numbered but 5,172,000. In the morning the relation was reversed, the venous count being 5,414,000 and the capillary 5,200,000. Similarly with the white cells the capillary count was higher than the venous in the evening but lower than the venous in the morning. This same relation appears in case No 2946, whose arterial tension and also pulse pressure was very low. Patients whose maximal diastolic pressure was less than 90 mm. of mercury did not show any such striking variations in cell counts. If we classify patients according to their systolic pressure, we have those whose systolic pressure exceeded 150 mm. and those whose systolic pressure was below 150 mm. of mercury. This group includes all those patients whose diastolic pressure exceeded 90 with four other cases and shows a higher capillary than venous count in the evening with reversal of this in the morning.

The high systolic pressure does not correlate with any constant variation in corpuscle counts. If, now, we attempt to compare the fall in blood pressure with changes in corpuscle counts, again we find no correlation. It seems clear that in these cases the variation in arterial tension does not influence the relation between the red counts in capillary and in venous blood. In this group of patients two features seem to disturb the relation between the corpuscle counts in capillary and venous blood; namely, low arterial tension and high diastolic pressure.

Cannon[3] found a high degree of capillary stasis in men suffering from traumatic shock. The capillary red count in sixteen of our twenty-seven cases was over six million. In eleven cases with low arterial pressure (systolic 52-102) and shock, differences of from 400,000 to 2,000,000 cells between the venous and capillary counts were observed. Cohnstein and Zuntz[4] noted that when blood pressure fell, after cutting the spinal cord, capillary stagnation occurred and the number of red cells in the venous blood fell rapidly (drop of 1,000,000 corpuscles in ten minutes). In our cases, the fall in pressure was probably too gradual to result in such marked changes. Case No. 2946 with blood pressure in the "shock zone" exhibited a degree of capillary stasis comparable with the cases of shock described by Cannon.

Mall and Welch[5] noted that when the mesenteric artery was in part occluded, with a fall in blood pressure, red cells jammed the venous system, then the capillaries; and finally hemorrhage occurred. This plugging of capillaries as a result of a stasis might result in less return of blood to the heart, of lessened output, and a lowered blood pressure. Such a vicious circle which would interfere with nutrition seems a possible explanation of some of the factors in gangrene in diabetic patients with low blood pressures. This same patient (No. 2946), although he did not have gangrene, exhibited in high degree the sluggishness and a lack of vigor in healing when he came to the hospital with hot water bottle burns of the feet. Another factor which may be compared in diabetes and in shock is the lowered oxygen content of venous blood. Grant[6] showed that the oxygen unsaturation of the venous blood of diabetics is much higher than that of normals. According to Krogh the capillaries open in oxygen want. This gives a slower flow and greater absorption of oxygen by the tissues. With the lowered blood pressure and the inadequacy of the oxygen delivery, there is a decrease of the metabolism; Cannon records a reduction of one-third in severe shock. In a group of forty-three diabetics, without thyroid disease, five patients with systolic blood pressure above 150 mm. Hg. had an average basal metabolism 8 per cent above normal. Thirty-two with systolic blood pressure between 100 and 150 had an average basal metabolism exactly normal; there were six cases with systolic blood pressure below normal standard whose average basal metabolism was 10 per cent below normal.

TABLE IV

THE EFFECT OF THE APPLICATION OF A TOURNIQUET ON THE CONCENTRATION OF RED CORPUSCLES IN VENOUS AND CAPILLARY BLOOD*

CASE NO.		RED CORPUSCLES				BLOOD PRESSURE MM. HG.
		BEFORE	AFTER			
			30 SECONDS	3 MINUTES	4 MINUTES	
		Millions	Millions	Millions	Millions	
Ba	Cap.	5.6	5.6	4.8		120/80
	Ven.	5.4		6.3		
McK	Cap.	4.3	3.5	3.8		80/60
	Ven.	4.3			5.3	
McK	Cap.	4.1	3.3		3.8	82/60
	Ven.	3.6			4.3	
Lou	Cap.	6.2	5.6	5.8		114/68
	Ven.	6.2		7.0		
Ha	Cap.	4.1	3.7		4.1	202/104
	Ven.	3.6			4.3	

*The tourniquet was applied tightly enough to cause distention of the vein but not to shut off the arterial supply.

Patient No. 3034 does not show the same relationship in spite of a low arterial pressure. This may be explained in part by the fact that she had had acidosis and had been given large amounts of fluid. The group of cases, with high diastolic pressure and capillary stasis in the evening and a change in this relation in the morning, illustrate another mechanism producing capillary stasis. Gisell (quoted by Cannon) showed that after a reduction of blood volume but before the blood pressure fell, there might be a fall in the oxygen content due to constriction of the peripheral vessels. We may reason that in

these cases of high diastolic pressure there is an abnormal tension of the small arterioles with a compensatory dilatation of the capillaries under the stimulus of greater oxygen need during the day time. During the night, with a fall in pressure, this stasis is in part or wholly dissipated.

In contrast with the effects of change in arterial pressure Table IV shows the effects on the concentration of red corpuscles produced by variations in the venous pressure. It is seen that when a tourniquet is applied around the arm tightly enough to prevent the venous return, marked variations in the red counts occur. At the end of thirty seconds the capillary count falls, and at the end of three minutes it tends to rise a little but still to be much lower than the concentration in the venous blood. The obvious explanation seems to be that the blood plasma leaves the venous vessels, giving a relative concentration in the veins. The reduction in capillary count may be due to dilatation of the capillaries and dilution.

The passage of plasma into the tissue spaces would seem comparable to edema occurring in heart failure with venous engorgement. One might expect a concentration of red cells in the capillary blood equal to that in the venous, on the theory that the increased venous pressure would produce a flow of plasma from the capillaries into the tissues. However, these five cases show clearly opposite effects in venous and capillary blood. It suggests also that a relation may exist between disease of the finer vessels and effects produced by change in venous pressure Thus, in the case of Mr. McK, who had marked edema with low blood pressure, the most marked reduction in capillary count was observed. This suggests that red cells may leave the capillaries in such cases, or else that certain of the capillaries under these conditions admit plasma but not corpuscles. In conditions of edema or of impending edema the capillaries or small veins may be peculiarly sensitive to changes in the venous pressure. At any rate, the subject merits further clinical study.

SUMMARY

1. The relative concentration of corpuscles in capillary and venous blood of twenty-seven diabetic patients in the morning and evening is compared.

2. Variations in arterial tension between these periods do not correlate constantly with any change in the corpuscle concentration.

3. In certain patients, with high diastolic blood pressure, the capillary count (red) was higher than the venous red count at night but lower in the morning. This reversed relation suggests capillary stasis at the end of the day and was more marked in patients with obvious vascular disease.

4. Increases in venous pressure produce the contrary effect; namely, high venous count and low capillary count.

5. It is suggested that further study is desirable. Comparisons of the relative concentration of corpuscles under varying conditions of arterial and venous pressure by means of the hematocrite should be made in cases of rheumatic heart disease, vascular hypertension and edema of cardiovascular origin.

mann reaction by the cattle blood hemolytic system and the sheep cell system. Their paper contains little of interest today, except their statement that between the positive reaction in syphilitic and that in probable nonsyphiltiic blood, there seemed to be a consistent difference in using the cattle blood system. In syphilis complement binding occurred only after the addition of organ extract, at least it was definitely strengthened by it, while in nonsyphilitic blood the lack of or addition of extract made no difference in the intensity of the fixation. They called an anticomplementary serum "autotrope serum." When sheep's blood amboceptor was used the autotrope serums reacted as syphilitic blood. When extract was added hemolysis was inhibited, and without extract more or less definite hemolysis occurred. The cattle blood system seemed to make it possible to distinguish a specific from a nonspecific reaction. The authors mention tuberculosis of the lungs and kidneys, pneumonia, carcinoma, myeloid leucemia and cardiac disease with compensation as nonsyphilitic diseases exhibiting this phenomenon of false fixation by so-called autotrope serum.

Hecht next discussed this subject of anticomplementary serum (eigenhemmendes Serum). He maintained that practically all blood has natural sheep amboceptor, that practically all blood has complement fixing substances, and that nearly all have complement. He believes that lack of balance of these substances causes fixation without antigen.

Ehrmann and Stern the next year quoted Ballner and von Decastello's work, but believed that the anticomplementary reaction might be characteristic of syphilis, as they stated that syphilitic serum in large quantities would fix complement without antigen. They stated that syphilitic serums reacting positively in quantities of 1 c.c. would frequently fix complement without organ extract, while nonsyphilitic serums did not show this phenomenon. Hecht controverted this statement by Ehrmann and Stern by asserting that he did not consider this reaction positive for syphilis. He showed that, in one case of lupus verrucosus, the anticomplementary reaction of the serum was stronger than in two cases of syphilis with anticomplementary serum, and that normal serum mixed with sheep's blood fixed complement in quantities of 1 c.c.

Only one other paper of these early writers need be mentioned, that of Trinchese, who reported a series of 7,000 Wassermann reactions and observed autoinhibition once in 500 serums, or in practically the same ratio as was observed in the Mayo Clinic in 1924. He stated that clinical experience has shown that autoinhibition in the original Wassermann reaction occurs only in syphilitic serums or in serums suspected of being syphilitic, and that it depends on the presence of antigen in the syphilitic serum.

It is seen by these references to the literature that the anticomplementary reaction of serum was early recognized and that there was from the beginning much difference of opin[illegible] what it meant. Differences in technic might [illegible] partly for the [illegible]ults. There are no reports in the literature [illegible] anticompleme[illegible]ctions with the Kolmer technic, other than [illegible]ished in the di[illegible] the p[illegible] referred to.

[illegible]sis of the nir[illegible]ction[illegible]r series has been made, and I [illegible]y that our co[illegible]nno[illegible] time throw much light on the

subject. While the number of reactions was ninety-six, the total number of patients was but fifty-eight, as there were many repeated tests. The serums of thirty-seven patients were anticomplementary one or more times. Not all of these patients in this group had spinal fluid examinations, but such spinal fluids as were examined were not anticomplementary. On the other hand, of the twenty-one patients whose spinal fluids showed one or more anticomplementary reactions, in no case did the serum give such a reaction, although several examinations of the blood of each patient were made. In other words, there was not a single instance of anticomplementary reaction of both blood and spinal fluid in the same patient. Whether this is of any significance cannot be determined in a small series. However, the anticomplementary reaction of spinal fluid has apparently attracted but little attention, according to other writers, although this condition must be met with by all serologists.

ANTICOMPLEMENTARY BLOOD SERUM AND SPINAL FLUID

There were thirty anticomplementary reactions in twenty-two cases diagnosed as nonsyphilitic, although it must be said that in some of these cases the likelihood of syphilis was not thoroughly investigated by the clinician, because there were no outward signs or history of the disease. The diagnoses of the noninfectious diseases in these twenty-two cases were: neurasthenia, two; menopause neurosis, one; psychoneurosis, one; general arteriosclerosis, one; achlorhydria, one; sacroiliac strain, one, and colloid goiter, one. The findings in these cases are apparently purely incidental and might possibly be attributed to some error in technic. There was also a group of cases of infection: conjunctivitis, one; retinitis proliferans, one; infectious hepatitis, one; chronic cholecystitis, one; arthritis, one; myocarditis, two; endocarditis, one; streptococcemia, one; and tubercular peritonitis, one. There were also three cases of malignant disease: myeloma, one; metastatic carcinoma, one; and lymphosarcoma, one. There was one case of splenic anemia.

From the foregoing list of diseases it may be definitely concluded that the reaction may occur rarely in patients in whom there is no history of syphilis and who are suffering from some other disease. The reaction occurred sixty-three times in thirty-three patients with syphilis. There were also three doubtful cases of anticomplementary reactions with a probable diagnosis of syphilis without syphilitic history. The reaction may then be considered suggestive of syphilis and I believe that a report of anticomplementary reaction should be considered by the clinician as of enough significance always to investigate thoroughly the possibility of syphilis. While many patients with syphilis in this series have had treatment of various sorts, there were others who most noticeably had not had treatment. The effect of treatment is not evident. The occurrence of the anticomplementary reaction can be illustrated in a report of two small groups of selected cases.

SELECTED CASES WITH ANTICOMPLEMENTARY BLOOD SERUM

CASE 1.—A man, aged forty-three, complained chiefly of painful joints for fifteen years. He had been married eighteen years, without children. His wife had syphilis. There was no definite history of syphilis, but he had had gonorrhea fifteen years before. The Kolmer test, March 15, 20 (two different samples of blood), 21, 22, and 24 (two

different samples of blood), 1924, was anticomplementary. The serum was sent to Kolmer, who reported it anticomplementary. The diagnosis was indeterminate but probably syphilis.

CASE 2.—A boy, aged twelve, complained chiefly of physical and mental weakness all his life. The father had had chancre on the lip four months before the birth of the patient, and the mother had had chancre on the genitalia two months before his birth. The Kolmer test on the serum had been 44 November 21, 1923, and was again 44 January 25, and May 2, 1924. The serum was anticomplementary April 2, and May 27, 1924. The spinal fluid had been 44444 November 26, 1923, and was 4444– January 28; 4442– April 3; 444–– April 30; 444–– May 14; and 444–– May 27, 1924. The diagnosis was cerebrospinal syphilis, juvenile taboparesis.

CASE 3.—A man, aged sixty, complained of having had a lump above the eye for one year. He had had syphilis twenty years before. With the Kolmer test the blood serum had been anticomplementary on four different occasions in 1923, and July 7, 1924. The diagnosis was tertiary syphilis.

CASE 4.—A woman, aged thirty-three, complained chiefly of having felt generally run down for several years. There was no definite history of syphilis. With the Kolmer test the blood serum was anticomplementary September 16, 18, December 22, and 23, 1924. The Kolmer test was 44 September 23, 24, and 25. The spinal fluid gave a negative reaction. The diagnosis was latent syphilis.

From the foregoing selected cases I have tried to show that in some instances it seems impossible to get anything but an anticomplementary reaction in repeating the Kolmer test with fresh serum inactivated the ordinary length of time. In other instances the anticomplementary reaction may be present one day and a few days later with a new titration of different reagents the reaction does not persist. However, the clearing of the control tube then makes the reaction very strongly positive.

SELECTED CASES WITH ANTICOMPLEMENTARY SPINAL FLUID

CASE 5.—A man, aged sixty, complained of confusion of ideas and forgetfulness, for an indefinite period. There was no history of syphilis although his wife had syphilis. The Kolmer test on the blood had been 44 July 11, August 10, 1923, and July 1, 1924. The Kolmer test of the spinal fluid was anticomplementary twice in 1923 and again July 2, 1924. The diagnosis was late syphilis of the central nervous system.

CASE 6.—A man, aged thirty-nine, complained of stomach trouble and gave a questionable history of syphilis. The Kolmer test of the blood was 44 July 28, and 31, 1924, but negative November 11. With the Kolmer test, the spinal fluid was anticomplementary July 31, September 25, October 9, and 23, 1924. (The spinal fluid has been anticomplementary once in March, 1925.) The diagnosis was late syphilis of the central nervous system.

CASE 7.—A man, aged forty-three, had complained of extreme nervousness for nine months. He had had a lesion on the penis fourteen years before, with no treatment. The Kolmer test of the blood was 44 January 2, 5, and May 22, 1924. The spinal fluid was anticomplementary January 5, and 4444 – May 22, 1924. The diagnosis was syphilis of the central nervous system, paresis sine paresi.

CASE 8.—A man, aged thirty-four, had had syphilis twelve years before with much treatment. The Kolmer test of the blood was 44 January 4, 8, 9, February 7, June 17, and July 2, 1924. The Kolmer test showed that the spinal fluid was anticomplementary January 9, 23, February 6, 20, June 18, July 18, 1924. The diagnosis was syphilis of the central nervous system.

The Nonne-Appelt test on all of these spinal fluids showed an increase in globulin, and the lymphocyte counts were all above normal.

It would seem, then, that an anticomplementary reaction with spinal fluid is a more likely indication of syphilis than when it occurs with serum. This may be more apparent than real, inasmuch as there are fewer negative controls since spinal punctures are not made as often as venipunctures in non-syphilitic cases. However, there is always a large proportion of spinal fluids in every day's run of Wassermann tests that give negative results. Paresis and taboparesis, has been the diagnosis in so many of these cases in which the spinal fluid has shown anticomplementary reaction, that the syphilologists in the Mayo Clinic are beginning to feel that the anticomplementary reaction in the spinal fluid may be an indication of this disease. In any event the number of cases observed is undoubtedly too small to make this statement of any positive value.

Several attempts were made to learn something of the nature of the anticomplementary substance. While none of these has proved of much worth, I shall present them briefly in order to indicate the difficulties encountered in making such a study. It is, after all, part of the whole story of what constitutes the basis for any reaction in immunity.

In Table I is reported a series of titrations to determine the amount of anticomplementary substance in various serums with which there was no hemolysis in the control tube in the ordinary quantity used, namely 0.1 c.c. It will

Table I

Titer of Anticomplementary Substance in Blood Serum

Complement in all tubes in double unit quantities

Number	Serum with antigen					Serum without antigen				
	0.1 c.c.	0.05 c.c.	0.025 c.c.	0.005 c.c.	0.0025 c.c.	0.1 c.c.	0.05 c.c.	0.025 c.c.	0.005 c.c.	0.0025 c.c.
1	++++	++++	++++	–	–	+++	–	–	–	–
2	++++	++++	+++	–	–	+++	+	–	–	–
3	++++	++++	++++	++++	++++	++++	++++	++++	–	–
4	++++	+++	–	–	–	++++	++	–	–	–
5	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
6	++	+	–	–	–	+	–	–	–	–
7	++++	++++	+	–	–	++++	+	–	–	–
8	++++	++++	++++	–	–	+++	++	–	–	–
9*	++++	++++	+++	–	–	+++	++	–	–	–
10	++++	++++	++++	++	+	++	+	–	–	–

*Same as No. 8, two days later

Table II

Titer of Anticomplementary Substance in Blood Serum

Fixing power in higher concentration of complement

Number	Serum with antigen*					Serum without antigen*				
	Concentration of complement					Concentration of complement				
	5X	4X	3X	2X	1X	5X	4X	3X	2X	1X
1	–	++	+++	++++	++++	–	–	–	–	++
2	–	–	++	++++	++++	–	–	–	++++	++++

*Quantity of serum in each tube, 0.1 c.c.

be seen that in only one instance did the titration of the anticomplementary substance exhibit the same degree of fixation as where the serum was used with antigen. Ruediger, in the discussion of Kolmer's paper, said that he was able to get rid of the anticomplementary substances by reheating at intervals of two or three days, or by running a control tube for every antigen tube, using the human serum in decreasing quantities, because in such cases the specific fixation will always occur in dilutions in which the anticomplementary property cannot be detected. This is evidently true in the small series that I was able to run. I found also that blood saved in the ice box for three days was no longer anticomplementary. Fresh serums may account, to some extent, for the anticomplementary reactions in this series.

In Table II, I have shown the results with two other serums that were titrated in another manner, namely, by determining the fixing power in higher concentrations of complement. I have done this several times when I have been interested in the amount of syphilitic antibody present in the serum, determining the quantity of complement that could be fixed with a constant quantity of patient's serum, rather than determining how little of the patient's serum would fix a constant quantity of complement. It is shown that the anticomplementary substance without antigen will not fix as high a concentration of complement as will the same serum when used with antigen.

TABLE III

TITER OF ANTICOMPLEMENTARY SUBSTANCE IN SPINAL FLUID

Complement in all tubes in Kolmer double unit quantities

NUMBER	SPINAL FLUID WITH ANTIGEN					SPINAL FLUID WITHOUT ANTIGEN				
	0.5 c.c.	0.25 c.c.	0.125 c.c.	0.0625 c.c.	0.03125 c.c.	0.5 c.c.	0.25 c.c.	0.125 c.c.	0.0625 c.c.	0.03125 c.c.
1	++++	++++	++++	++++	+	++++	+++	–	–	–
2	++++	++++	++++	+++	–	+	+	–	–	–
3	++++	++++	++++	–	–	++++	+	–	–	–

TABLE IV

TITER OF ANTICOMPLEMENTARY SUBSTANCE IN SPINAL FLUID

Higher concentration of complement

NUMBER	SPINAL FLUID WITH ANTIGEN* CONCENTRATION OF COMPLEMENT					SPINAL FLUID WITHOUT ANTIGEN* CONCENTRATION OF COMPLEMENT				
	5X	4X	3X	2X	1X	5X	4X	3X	2X	1X
1	+	++++	++++	++++	++++	0	++	+++	++++	++++
2	Not enough fluid					–	++	+++	++++	++++
3	+	++++	++++	++++	++++	–	–	–	–	+++

*Quantity of spinal fluid in each tube, 0.5 c.c.

Tables III and IV show in like manner the titer in anticomplementary substance as found in spinal fluid. In Table III the titration of spinal fluid is against a constant quantity of complement both with and without antigen; in Table IV a constant quantity of spinal fluid, namely, 0.5 c.c., was used, and the degree of fixation with higher concentrations of complement up to five times the normal amount, both with and without antigen is recorded.

Hecht's suggestion regarding the cause of "autoinhibition" prompted an attempt to titrate the amount of complement in anticomplementary serum. Table V shows the result of one of these experiments. A criticism of the result is apparent. While there was no hemolysis with any quantity of anticomplementary serum, *the conclusion cannot* be *drawn that* there is no complement present in such serum, inasmuch as there would be fixation of complement by the anticomplementary substance; no satisfactory experiment could be devised for demonstrating whether or not an anticomplementary serum really contains complement.

It was suggested by Trinchese that the property is *due to the presence of* antigen in the serum. Table VI shows a small series in which the anticomplementary serum was titrated for antigenic properties. The control tubes with-

TABLE V

COMPLEMENT TITRATION OF HUMAN SERUM

SERUM	SALT SOLUTION 0.0 C.C. SERUM (1/10) 1.0 C.C.	SALT SOLUTION 0.2 C.C. SERUM (1/10) 0.8 C.C.	SALT SOLUTION 0.4 C.C. SERUM (1/10) 0.6 C.C.	SALT SOLUTION 0.6 C.C. SERUM (1/10) 0.4 C.C.	SALT SOLUTION 0.8 C.C. SERUM (1/10) 0.2 C.C.	REMARKS
Anticomplementary	O	O	O	O	O	Amboceptor 1/2000
Syphilitic	H	H	H	±	O	0.5 c.c. each tube
Negative	H	H	H	H	O	0.5 per cent sheep cells
Negative	H	H	H	H	O	0.5 c.c. each tube
Negative	H	H	H	H	O	Incubation one hour
Negative	H	H	H	H	O	

H = hemolysis, ± = incomplete hemolysis; O = no hemolysis.

TABLE VI

TITRATION FOR ANTIGENIC PROPERTY OF ANTICOMPLEMENTARY BLOOD SERUM

SERUM*	ANTICOMPLEMENTARY SERUM				
	0.1 c.c.	0.05 c.c.	0.025 c.c.	0.005 c.c.	0.0025 c.c.
Control (no serum added)	++++	++++	–	–	–
1. Syphilitic	–	–	–	–	–
2. Syphilitic	–	–	–	–	–
3. Syphilitic	–	–	–	–	–
4. Syphilitic	–	–	–	–	–

*0.1 c.c. in each tube.

TABLE VII

TITRATION FOR ANTIGENIC PROPERTY OF ANTICOMPLEMENTARY SPINAL FLUID

	ANTICOMPLEMENTARY SPINAL FLUID				
	0.5 c.c.	0.4 c.c.	0.3 c.c.	0.2 c.c.	0.1 c.c.
Control (no serum added)	++++	++++	++++	+++	–
Syphilitic spinal fluid, 0.5 c.c.	++++	++++	++++	+++	–
1. Syphilitic serum, 0.1 c.c.	–	–	–	–	–
2. Syphilitic serum, 0.1 c.c.	–	–	–	–	–
3. Syphilitic serum, 0.1 c.c.	–	–	–	–	–
4. Syphilitic serum, 0.1 c.c.	–	–	–	–	–
5. Syphilitic serum, 0.1 c.c.	–	–	–	–	–
Pooled syphilitic serum, 0.1 c.c.	–	–	–	–	–

out syphilitic serum showed that this particular serum was anticomplementary in quantities of 0.1 c.c. and 0.05 c.c., but when syphilitic serum was added there was no fixation of complement.

In Table VII the results of titration of anticomplementary spinal fluid for antigenic property are recorded. This spinal fluid was anticomplementary in quantities down to 0.2 c.c. When syphilitic serums were added there was no fixation of complement. However, when a syphilitic spinal fluid was added to each tube in 0.5 c.c. quantities, there was the same degree of fixation that had been manifested by the anticomplementary spinal fluid when used alone. It is doubtful whether this is an evidence of antigenic action. Fixation is probably due to the anticomplementary substance in the spinal fluid, uninfluenced by the addition of syphilitic spinal fluid. It is a little difficult to conjecture, however, what has happened to the anticomplementary fluid when syphilitic serum is added to the various tubes and no fixation of complement occurs. Further study must be made of this type of reaction to really make clear the significance of this phenomenon.

At present a study is being carried on in our laboratory comparing precipitation tests on serums that have been shown by the Kolmer test to be anticomplementary. As complement plays no part in this test it might be argued that an anticomplementary reaction could not occur, although it must be admitted that the same substance that is fixing complement, whether with or without antigen, may also be responsible for the flocculation phenomenon described by Kahn, Vernes, Meinecke, Sachs-Georgi, and other advocates of precipitation tests for syphilis.

SUMMARY

An anticomplementary reaction of fresh serum, or spinal fluid, occurs with the Kolmer modification about twice in each thousand tests. This reaction is not specific for syphilis, although it occurs more often in syphilitic than in nonsyphilitic cases. In fact, its occurrence with spinal fluids may be indicative of severe syphilitic involvement of the central nervous system. The nature of the anticomplementary substance has not been fully determined. It does not seem to be due to natural antigen in the serum, or spinal fluid itself. It is transient in character in some cases, and in others is found constantly. Quantitatively it is not as active as is the complement-fixing property of syphilitic serum when antigen is added. Flocculation tests on anticomplementary serums may throw some further light on this interesting subject.

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A STANDARDIZED WASSERMANN REPORT*

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THE purpose of this paper is to try to induce laboratory workers to state the results of their Wassermann tests in simpler language which will be better appreciated by clinicians.

The Wassermann test is made differently in almost every laboratory. Different degrees of accuracy and sensitivity result, each worker having his own idea as to the desirable degree of sensitivity for his results to be diagnostic of syphilis. The same worker may argue against the reporting of complete fixation as positive on the grounds that in stating that the test is positive he would be making an improper interpretation for the clinicians.

It is practically impossible to cause the majority of workers to adopt any definite technic because of differences of opinion and technical facilities. Near one extreme we have those diminishing few who, apparently from choice, attempt to fix complement by plain syphilitic liver antigens approximately in one hour at 37° C., and near the other extreme we have a large group of those with good technical facilities who do their fixation with purified lipoids for approximately twenty hours at temperatures lower than 8° C.

The reports to the clinician are given in various terms which suggest accuracy, probably the commonest terminology is plus-four as indicating complete fixation, plus-three as about three-fourths fixation, plus-two as one-half fixation, and plus-one as one-fourth fixation. The clinician is led to believe by such a report that a plus-two serum is necessarily just half as strong in Wassermann bodies as a plus-four serum which apparently contains the maximum amount of Wassermann bodies. The clinician is told of the relatively unimportant distinctions between a serum which is plus-three and one which is just plus-four, but he is deceived by being given the same report of plus-four on two serums, one of which may be sixteen or more times as strong as the other. If the clinician is told merely that both of these plus-four serums are positive he does not receive any suggestion that they are of exactly the same strength.

In the past, when the weak antigens produced less than complete inhibition in secondary syphilis, there was a greater difference than now between a plus-two and a plus-three serum and it may have seemed of some value to report such differences. No difference would be noted with such serums tested today by the more sensitive methods (except by quantitative tests) as they both would show complete inhibition. There is also less difference today between plus-one and plus-two serums. This difference continues to grow less with each increase in sensitivity.

*Read before the Fourth Annual Meeting of the American Society of Clinical Pathologists, Philadelphia, Pa., May 20-23, 1925.

This system of recording complete fixation in a single tube by four-plus is a common method in this country in private laboratories but, in the navy, a system is used in which two-plus signifies a complete reaction. Then there are other methods particularly in state laboratories where the results in two dilutions of serum are added together; plus-two, or complete fixation, in two tubes is recorded as plus-four, and a plus-two might mean plus-one in each of two tubes, or complete fixation in one tube with no fixation in the other.

These various hieroglyphics are understood by the individual serologist; they are useful in recording results according to his own private formula, but they should not be allowed to mislead the clinician.

The ordinary qualitative report is of sufficient assistance to the clinician in discovering the presence of syphilis. A report of a negative, positive, or doubtful result is all that is necessary for that purpose. Partial inhibition of hemolysis should be reported as doubtful; probably only complete fixation should be reported as positive. The clinician should not be given any spuriously quantitative reports. The only quantitative statement which should have any place on a qualitative report should be one giving a coefficient of sensitivity. It might be stated on the report that the method used is positive to a certain number of "A.S.C.P." or perhaps "Wassermann" units. Then the clinician will be better able to evaluate the evidence of a negative Wassermann report.

A quantitative test should be available to those who are giving treatment. An arbitrary numerical standard should be adopted by the American Society of Clinical Pathologists to be called the "A.S.C.P." unit which should correspond approximately in different laboratories.

Without going into more detail than is necessary to show the feasibility of this plan, I shall outline a simple procedure by which it is possible to standardize the results of the test. The details should be determined by a committee of this society.

Strongly positive serum is dried by spontaneous evaporation at room temperature in watch crystals. One-half a milliliter of serum is placed in each of six or more watch crystals. After the serum has dried, the crystals are stored in a desiccator for two weeks or more. The serum should be of such strength that it will give a positive reaction in a dilution of one in four or more with a certain definite standard method. One watch crystal of this dried serum is redissolved in the calculated amount of distilled water and salt solution to make a one in four dilution and used as though it were pure serum. Then this diluted serum is tested quantitatively in the same and greater dilutions to determine which dilution gives complete fixation according to a definite standard technic. The coefficient of sensitivity of this standard method naturally would be considered as 1.

The dilution which gives just complete fixation shall be considered arbitrarily as being an undiluted standardized four unit serum. In other words, the standard method gives complete fixation with a serum containing just four Wassermann units. A more sensitive method might give complete fixation with a further dilution of the four unit serum to one in four. Then

the more sensitive method would have a coefficient of sensitivity of 4 as it would be four times as sensitive as the standard method with a coefficient of 1.

If standardized four unit serum is used, the simple rule for obtaining this coefficient is to divide the coefficient of the standard method (1) by the further dilution of the serum necessary to obtain just complete fixation with the new method (or by the proportionate reduction or increase in dosage from the usual in the new method).

Example 1: A new method requires further dilution of standard serum to 1 in 4. $1 \div \frac{1}{4} = 4$. Therefore the coefficient of sensitivity is 4. This coefficient shows the new method to be four times as sensitive as the standard method.

Example 2: The usual dose of serum in a new method is 0.5 c.c. of serum, but instead of further dilution of the standard serum being used a dose of 0.125 c.c. is found to give just complete fixation.

$$1 \div \frac{0.125}{0.5} = 4.$$

Example 3: A new method does not give complete fixation with 0.5 c.c. the usual dose used, but requires double that dose or 1 c.c.

$1 \div \frac{1}{0.5} = 0.5$ Therefore the coefficient of sensitivity of the new method is 0.5. The new method is half (0.5) as sensitive as the standard method.

If serum of known strength other than four unit strength is used, the same rule will apply, but the result must be multiplied by the proportion existing between the strength of the standard serum and of the serum to be used.

Example: Strength of serum used 32. Usual dose of serum in new method 0.5 c.c., required dose 0.125 c.c.

$$1 \div \frac{0.125}{0.5} = 4.$$

$4 \times \frac{4}{32} = 0.5$, the coefficient of sensitivity.

Any method giving constant results, when its coefficient of sensitivity has been determined can be used for standardizing serum for standardizing other methods. To find the strength of a serum in units, the coefficient of sensitivity is divided by the dilution necessary (or by the usual dose into the dose necessary) to just give complete fixation, and the product is multiplied by 4. The product is multiplied by four, because a serum is considered to be of 1 unit strength when it contains one-fourth the amount of Wassermann substance necessary to give complete fixation with a method with a coefficient of 1. The serum is then diluted as indicated to make it a standard four unit serum.

Example 1: Coefficient of method is 2. Necessary dilution of serum is 1 in 4. $(2 \div \frac{1}{4}) \times 4 = 32$. Therefore the unknown is a 32 unit serum. This serum if diluted to 1 in 8 would be a standard four unit serum.

Example 2: Coefficient is 2. Usual dose of serum 0.5, necessary dose 0.125.

$$(2 \div \frac{0.125}{0.5}) \times 4 = 32.$$

Where there is any local difficulty different laboratories can easily check results by exchanging standardized dried serum.

The method used for standardizing should be one which is quite simple, available to workers with limited facilities, and one which eliminates as far as possible any personal equation. A serum is diluted at least one in four and greater dilutions are made until some dilution gives just complete fixation with the following technic. That liquid is considered as containing four units, is called the standard four unit serum, and is used for standardizing any other method.

In the standardizing method the serum is inactivated for one-half hour at 56° C. The dosage of the standard four unit serum is 0.1 c.c.

The complement is used on the day the test is made but aged by being placed in the 37° C. incubator for one hour before separating the serum. After separating the serum it is diluted one in twenty, and 0.5 c.c. is used as the dose. A definite dose of complement is used unless the complement is found to be quite inactive when it is discarded. The definite dose is used instead of varying it by means of the hemolytic activity. In my opinion attempting to estimate the fixability of complement by the hemolytic activity will give more variability than the use of a fixed dose of guinea pig serum. This is one of the details which should be determined by a committee and I am suggesting this method in some detail merely as a framework and not to provoke controversy.

The antigen is next added in a dose of 0.2 c.c. of a one in ten dilution. The antigen should be prepared after the method of Neymann and Gager.[1]

Pulverized heart muscle (Difco brand is suitable) is completely extracted with ether to remove cholesterol and certain substances of variable composition. The heart, free from ether, is added to absolute alcohol in the proportion of 20 grams of heart to 100 c.c. of alcohol. This mixture is placed in the incubator at 37° C. for one week. The alcohol is poured off or filtered off and cholesterol is added to make a 0.2 per cent solution. This antigen, because of the low cholesterol content, is diluted slowly.

Fixation is accomplished by leaving the tubes in a water-bath at 37° C. for one hour.

Sheeps corpuscles, not over twelve hours' old, are next added in a dosage of 0.2 c.c. of a 6.25 per cent suspension.

Two units of amboceptor contained in 0.2 c.c. of solution are next added and the tubes shaken and returned to the water-bath. The readings are made as soon as the cells have settled sufficiently, for the total absence of hemolysis in some tubes to be clearly visible.

Some other Wassermann technic may be found more constant in its results or otherwise more desirable, but the method suggested will serve the purpose until a better one can be determined upon. Slight variations in numer-

[1]Neymann and Gager: Jour. Immunol., 1917, ii, 573.

ical results can be eliminated if necessary by repeating the test a number of times on the serum to be standardized and averaging the results.

The routine test for diagnosis should be the qualitative test, the result of which should be given as negative, doubtful, or positive and accompanied by the coefficient of sensitivity for the method used.

The routine test for observing the effects of treatment should be the quantitative test, the result of which should be expressed in "A.S.C.P." units.

THE PRESENT STATUS OF KOLMER'S COMPLEMENT-FIXATION TEST FOR SYPHILIS AS ESTABLISHED BY A CRITICAL COMPARISON WITH NUMEROUS OTHER METHODS*

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THE value of any new laboratory procedure or any modification of a method already in use can only be ascertained by comparative trial, and the more critical the comparison and the more extensive the trial the more significant are the ultimate conclusions.

Of all the methods which have been proposed for the adaptation of the complement-fixation test to the serologic diagnosis of syphilis there are none which have been subjected to comparative, critical, and even at times hypercritical, trial than that proposed by Kolmer.[1]

It is the purpose of this communication to summarize succinctly the reports thus far made upon this technic with a view to ascertaining the present status of the method with particular reference to (a) delicacy and sensitivity; and (b) the specificity of the reactions obtained with it.

It is impossible in the limits of the time allowed to present a full consideration of the minutiae of the various reports nor is it deemed necessary as a detailed analysis of the data to be here briefly summarized will be presented in another place.[2]

In Table I are gathered the investigations upon the analysis of which this report is based, comprising a total of 88,539 reactions conducted and reported upon by 35 different workers.

It is quite probable that no other technic has been subjected to comparative investigation in so large a series and the value and significance of the findings so obtained are enhanced by, if indeed they do not arise in essence from, the very multiplicity of the workers and the variety of methods with which the Kolmer test was compared.

The mere collection of a large number of tests is not of value per se; their significance and the value of the conclusions to be drawn from them in this instance is dependent upon the following factors and conditions forming an integral part of the investigations and their conjoint results:

*From the Laboratories of the Atlantic City Hospital.

Read before the Fourth Annual Meeting of the American Society of Clinical Pathologists, Philadelphia, Pa., May 26, 1925.

TABLE I

TOTAL REACTIONS REPORTED BY WORKERS INVESTIGATING KOLMER'S TEST

AUTHOR	NUMBER OF TESTS
Cohen and Haythorn	1,400
Dutton and Thompson	501
Harper and Curtis	120
Hartman and Reyner	6,947
Hartman and Reyner	20,307
Irvine and Stern	5,162
Johns and Jones	500
Kellogg	9,307
Kellogg, Wells, and Beck	506
Kilduffe	1,014
Kilduffe	2,000
Kilduffe	104
Kilduffe	2,180
Kilduffe	300
Kolmer	355
Kolmer and Denney	159
Lorenz and Bleckwenn	997
Maynard	806
Ottenberg and Wisler	204
Palmer	2,500
Palmer and Gibb	363
Palmer	2,502
Rockwood and Sanford	500
Sanford	4,740
Butler	16,772
Goodhue	700
Schamberg and Greenbaum	3,000
Schamberg and Klauder	3,000
Shivers	320
Smith	94
Total	88,359

1. In each instance the Kolmer technic was compared with a method regarded by the authors as reliable and sensitive and thoroughly evaluated by past experience.

2. All the investigations were made and controlled under strictly experimental conditions in that every effort was made to collect full clinical data on the tested cases, particularly where there was disagreement between the methods used.

3. A sustained effort was made to subject the Kolmer technic to stringent trial in those conditions generally accepted as giving false positive reactions.

4. With the exception of Dr. Kolmer (reporting 459 reactions of the total), none of the investigators was associated with the development of the technic and, therefore, were not prebiased in its favor.

The adaptability of the Kolmer technic to and its conformity with the requirements of the laboratory and the serologist for a standard method have been fully discussed elsewhere.

As a standard method must be adapted to varied conditions from the ideal to the relatively vile, the incidence of anticomplementary reactions in the Kolmer test is of practical interest. A personal experience in over 10,000 tests shows an incidence of only 0.7 per cent and the remarkable freedom of the method from anticomplementary reactions is commented upon by all observers.

It cannot be too strongly emphasized that to obtain the excellent results possible with the Kolmer technic *it must be used exactly as described by the author to the minutest detail and that deviations, no matter how apparently minor, will be reflected in a diminution of the sensitivity and delicacy of the test.*

By reason of the long period of primary incubation the technic is not economical in time and objection has been made because of this fact.

Kolmer has stated in his original communication that while, if compulsory, a four-hour incubation may be used instead of the overnight period, the delicacy of their action is affected.

Because some may hesitate to adopt the method because of the delay in reporting, and to ascertain the difference referable to variations in the primary incubation period, I have recently studied a series of 300 serums tested by the quantitative technic but with differing incubation periods as follows:

1. Ice chest four hours at 6°-10° C.
2. Ice chest 16-20 hours at 6°-10° C.
3. Ice and brine bath four hours at 6°-10° C.

The results of the investigation, which have been reported elsewhere,[3] quite definitely demonstrated that not only was the quantitative character of the test more clearly brought out by the longer incubation period, but, what is even more important, while the number of positive reactions detected by both four-hour periods was practically the same, there were 9.6 per cent more positive reactions—mainly one and two tube fixations—detected by the overnight period.

The overnight period of primary incubation, therefore, is a valuable, integral, and important part of the technic and cannot be disregarded except at the expense of delicacy.

This being true, objections based upon this point cease to be valid.

Of particular interest are the conclusions formed as to the specificity of the Kolmer test.

Specificity implies that positive reactions shall not be obtained in conditions other than syphilis. As the complement-fixation test as applied to syphilis is not a true antigen-antibody reaction in a biologic sense, all that can be hoped for is a *relative* specificity.

The practical specificity of the reaction in syphilis, therefore, depends upon whether or not the lipotropic reagin responsible for the reaction in syphilis may be present in other conditions also. It is known that it exists in one other condition, frambesia or yaws, possibly because of the close biologic relationship existing between its cause, the Spirocheta pertenuis, and the Spirocheta pallida.

In Table II are gathered the various conditions other than syphilis in which nonspecific reactions have heretofore been obtained and which were investigated by the Kolmer test.

Large series of these conditions are neither easily nor rapidly obtained for obvious reasons; nevertheless, the total number possesses a definite significance and indicates that false positive reactions are extremely rare with the Kolmer technic *if, indeed, they occur at all.*

ADVERSE REPORTS

There were no adverse reports in the series under consideration, all workers agreeing that the method was sensitive, reliable, did not give false positives and but few anticomplementary reactions and that there was a high percentage of agreement with the clinical findings.

There are only two definite criticisms: (1) The occurrence of a definite number of false negative reactions; and (2) the fact that the Kolmer method requires more time, more tubes, and a little more labor.

The first of these is valid and demands study and has already been referred to.

It is difficult to believe, however, that any serologist would seriously urge as a valid objection to the adoption of an efficient, superior and specific test the fact that it was a little more time-consuming or a little more laborious. It is much more likely and easier to believe that this objection is really an indirect expression of a reluctance to cast aside a familiar method for a new one; certainly an objection based upon such grounds would be difficult and embarrassing to uphold.

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COMPARISON OF RESULTS WITH KOLMER-WASSERMANN METHOD AND KAHN PRECIPITATION TEST*

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In a paper[1] published some months ago we showed the results found in subjecting 2000 sera to the Kahn precipitation test, using his latest modification,[2] and to a Wassermann technic run with plain alcoholic antigens and with an 18-hour ice-box fixation period.

In that investigation we obtained an absolute check of 89.5 per cent with both tests and when the ++++, +++ and negative results were grouped together we found what we called a "diagnostic check" in 90.5 per cent of the cases.

There were 105 cases where one method showed a reaction of diagnostic strength (++++ or +++) while the other was of lesser degree and these cases were grouped as follows:

Wassermann ++++ or +++,	Kahn 0	10 (0.5 %)
Wassermann ++++ or +++,	Kahn +	19 (0.95%)
Wassermann ++++ or +++,	Kahn ++	30 (1.5 %)
Wassermann more strongly positive than Kahn		59 (2.95%)
Kahn ++++ or +++,	Wassermann 0	16 (0.8 %)
Kahn ++++ or +++,	Wassermann +	13 (0.65%)
Kahn ++++ or +++,	Wassermann ++	17 (0.85%)
Kahn more strongly positive than Wassermann		46 (2.3 %)

Nine of the 10 Wassermann-positive, Kahn-negative cases gave definite histories of syphilis with treatment while among the 16 Kahn-positive, Wassermann-negative cases, 12 were definitely syphilitic; with 3 we were unable to obtain any data and 1 was probably a false positive.

Having convinced ourselves of the superiority of the Kolmer method over our former technic we adopted it as our standard and the present series of 1600 cases was tested by the Kolmer method in comparison with the Kahn.

In order to establish some basis of comparison in reporting the results with the two tests we gave a value of 0 to 4 to the reaction obtained in each tube of the respective sets (3 tubes in Kahn and 5 in the Kolmer), and then arbitrarily assigned the following values to the sum total of the results in all the tubes of each set.

+	= Total of 1 to 2 with Kolmer	= Total of 2 to 4 with Kahn
++	= Total of 3 to 6 with Kolmer	= Total of 5 to 7 with Kahn
+++	= Total of 7 to 10 with Kolmer	= Total of 8 to 10 with Kahn
++++	= Total of 11 to 20 with Kolmer	= Total of 11 to 12 with Kahn

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For example, a Kolmer reading of 4—3—1—0—0 giving a total of 8 units we called +++, while a Kahn reading of 2—3—4, we also graded as +++.

We followed the exact details of the Kolmer method, except that we added the salt to the antigen in making our antigen dilution, while the only variation from the Kahn was in using a 15-minute inactivation period instead of 30, and in shaking the serum antigen mixture for four minutes by hand instead of for two minutes in a shaking machine, as we have not found that the longer inactivation period made any difference and Kahn himself advocates a two to four minute shaking period.

In this series of 1600 cases we got an absolute check in 1471 or 91.9 per cent, and by grouping the ++++, +++ and negative reactions together got what we called a "diagnostic check" in 1501 or 93.8 per cent.

There were 59 cases showing considerable divergence in results, ranging from negative with one test to ++++ with the other, practically all being treated cases.

These cases showed the following.

Wassermann ++++ or +++,	Kahn 0	5 (0.31%)
Wassermann ++++ or +++,	Kahn +	11 (0.69%)
Wassermann ++++ or +++,	Kahn ++	11 (0.69%)
Wassermann stronger than Kahn		27 (1.68%)
Kahn ++++ or +++,	Wassermann 0	18 (1.1 %)
Kahn ++++ or +++,	Wassermann +	3 (0.19%)
Kahn ++++ or +++,	Wassermann ++	11 (0.69%)
Kahn stronger than Wassermann		32 (2.0 %)

The great significance of our findings lies in the fact that there were five cases frankly negative with Kahn and strongly positive with the Wassermann and 18 where the opposite result occurred. Had dependence been placed on only the one method a certain percentage of positive results would have been missed.

The Wassermann-positive, Kahn-negative cases were:

1. 1478—old latent lues—last treatment 5 years ago.
2. 1465—chancre 5 weeks duration.
3. 1454—multiple gumma of leg
4. 1999—old treated case—has severe diabetes.
5. 2071—latent lues—treated 2 years ago

The Kahn-positive, Wassermann-negative cases were:

1. 969—last treatment 7 weeks ago.
2. 740—last treatment 8 days ago.
3. 1171—last treatment 1 month ago.
4. 1026—last treatment 3 months ago.
5. 1460—last treatment few months ago.
6. 1821—last treatment few months ago.
7. 1573—old treated case.
8. 2193—old treated case.
9. 2314—old treatment case.
10. 979—old treated case.
11. 1723—chancre 7 to 10 days.
12. 1147—child. No history or symptoms
13. 1587—no history or symptoms. Severe diabetes.
14. 1613—colored. No history or symptoms.
15. 1554—history not known.
16. 1181—history not known.
17. 1427—physician recovering from glandular fever. No history or symptoms.
18. 1625—physician's wife. Has early P. A. No history or symptoms.

These 18 cases comprise 6 recently treated, 4 old treated cases, 1 early chancre, 3 with no definite history of symptoms as determined by the attending physician, 1 with no available data and 2 where syphilis could presumably be excluded.

TABLE I

SUMMARY OF 1600 KAHN AND KOLMER TESTS

WASSERMANN

Kahn	++++	+++	++	+	0
++++	223	29	10	2	10
+++	7	11	1	1	8
++	7	4	5	1	11
+	7	4	5	1	14
0	2	3	1	2	1231

We still find that a not inconsiderable number of sera give weak precipitates at times, consequently we do not attach much importance to ± or + reactions, but where frankly positive results are obtained we feel that the patient should be subjected to further study.

The simplicity of the test and the rapidity with which the reactions may be read make it an ideal check on the Wassermann method, and we feel a much greater confidence in the correctness of our results where a serum is subjected to both tests than we formerly had in depending on the Wassermann set up alone.

The antigen-salt mixture, however, is an extremely unstable body and careful controls are just as essential as they are in the fixation method.

SUMMARY

Examining 1600 sera we obtained a practical check in 93.8 per cent of the cases.

The divergence between the two tests lies almost wholly among the treated cases.

The Kahn precipitation test furnishes an ideal check for the Wassermann reaction and will "pick up" a certain small percentage of positive results which may be missed by the older method. On the other hand, certain cases may show a positive Wassermann reaction and a negative Kahn.

To obtain the greatest accuracy possible all sera should be subjected to both the Kahn and Wassermann methods and where the results differ radically further study of the case is indicated.

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THE KAHN PRECIPITIN TEST AS COMPARED WITH THE KOLMER COMPLEMENT-FIXATION TEST*

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SINCE Kahn, in 1922, proposed his flocculation reaction for syphilis, there have appeared numerous reports supporting his contention that his test compares very well with the Wassermann test. The present report is based on a comparative study of 2540 consecutive sera tested with Kolmer's quantitative system and Kahn's latest modification of his own precipitin reaction. Forty of these are not included in this report since full clinical data was not obtainable. The sera were from patients admitted to the St. Joseph Hospital, the Epworth Hospital and the United States Public Health Clinic. A very large percentage of this material is made up of sera obtained from patients under treatment for syphilis, of whom a complete record of the progress following treatment at various intervals is available.

The technic employed for the Wassermann test is the standardized quantitative complement-fixation test described by Kolmer. This test was followed in every detail with the single exception, that the sera were inactivated for 20 minutes instead of 15 minutes. The precipitin reaction used is that described by Kahn, in which the ether extraction for the antigen is carried out at room temperature by four consecutive washings of the powdered muscle at ten-minute intervals with 100, 75, 75, 75 c.c. of ether respectively. The subsequent alcoholic extraction is carried out for three days at room temperature. Various amounts of stock alcoholic extract, sufficient to last about a month, were fortified by the addition of 6 mg. of cholesterin per c.c. The unit of each batch of cholesterinized antigen was determined by titration and 0.05 c.c. and 0.025 c.c. amounts were used in the test. These quantities were found sufficiently satisfactory and accurate for routine work, instead of using 0.05 c.c., 0.025 c.c. and 0.0125 c.c. as advocated by Kahn. The readings were made after overnight incubation at 37° C., after adding 0.5 c.c. of normal saline. It must be emphasized, in this connection, that overnight incubation is not necessary. The original technic was adhered to in order to complete the series herein reported. A second series already in progress using 15 minutes water-bath incubation is proving to be even superior to overnight incubation. The scale used is as follows:

Precipitate with one or more large clumps = 4 +
A large flocculent precipitate = 3 +
Medium size flocculi = 2 +
Fine granules visible to the naked eye and not soluble by shaking = 1 +
No perceptible precipitate negative.

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When referring to the Kolmer quantitative fixation test, the term weak-positive includes all reaction showing 50 per cent or less complement fixation in the first two tubes. Strongly positive includes all reactions showing complement fixation of more than 50 per cent in two or more tubes. In the Kahn test 1+ and 2+ are considered weakly positive and 3+ and 4+ strongly positive. This terminology, of course, is not that described by either Kolmer or Kahn and I have used it because of its brevity.

Tables I, II and III represent a summary of the results obtained.

TABLE I

Kolmer strongly positive and Kahn strongly positive	1685 or 67.4%
Kolmer negative and Kahn negative	540 or 21.6%
Kolmer weakly positive and Kahn weakly positive	92 or 3.68%
Total agreement in type and degree of reaction	2317 or 92.68%

TABLE II

Kolmer strongly positive and Kahn weakly positive	53 or 2.12%
Kolmer weakly positive and Kahn strongly positive	28 or 1.12%
Total agreement in type but disagreement in degree of reaction	81 or 3.24%

TABLE III

Kolmer strongly positive and Kahn negative	21 or 0.84%
Kolmer weakly positive and Kahn negative	27 or 1.08%
Kahn strongly positive and Kolmer negative	22 or 0.88%
Kahn weakly positive and Kolmer negative	32 or 1.28%
Total disagreement in reaction	102 or 4.08%

Table I shows that 2317, or 92.68 per cent, of the sera reacted in complete agreement both in type and degree of reaction, while 81 or 3.24 per cent vary only in intensity of reaction as shown in Table II. This phase is very interesting and I have noted that it occurred in my series only in patients who had received treatment. These sera, when rechecked, gave practically always identical results, thus excluding technical error. Rochstraw and Malcom state that this disagreement in degree of reaction never occurred in their experience. However, the total per cent of agreement closely checks with them.

Table III represents a group of 102, or 4.08 per cent, of the sera in which there is a complete disagreement. The 21 sera that were strongly positive with Kolmer and negative with Kahn, were from patients under treatment for lues. Fourteen of these yielded similar results on rechecking, while the other seven could not be repeated as the patients were ambulatory and their whereabouts unknown. Of the 27 sera in which the Kolmer test was weakly positive and Kahn negative, 20 were from patients under treatment from one to two years, 5 had a history of primary lesion ten years or more before and had received very little treatment, and two had no history or clinical evidence of syphilis. The reaction in these last two cases was 2, 1, and 2, 2, respectively. Of the 22 strongly positive Kahn and negative Kolmer, twenty were from patients under treatment for lues, one had a history of a primary infection fifteen years before with insufficient treatment and symptoms of tabes dorsalis, while

one had no clinical evidence of syphilis, although there was a history of exposure. Finally, of the 32 weakly positive Kahn and negative Kolmer, ten had been under treatment for syphilis and discharged, nine were still under treatment and thirteen had no history or clinical evidence of syphilis.

In connection with these thirteen apparently false weakly positive sera, it must be emphasized that I have used overnight incubation which, while it renders the precipitates more conspicuous, does, however, increase the possibility of error.

SUMMARY

In summing up, it is evident that the two tests run parallel in about 96 per cent of the sera. Both tests occasionally render a false negative which is usually picked up by one or the other when the two tests are run parallel. When we consider that antigens vary in polyvalance, this discrepancy is to be expected by any two tests employing different antigens. The Kahn test, however, has several points of advantage, namely, it excludes the hemolytic system which does away with the necessity of expensive equipment, so that the test is available to small institutions maintaining a moderately equipped laboratory; anticomplementary reactions are eliminated and may in the future solve the problems associated with this phenomenon; and lastly, it can be utilized to a great advantage in testing donors for emergency transfusions. One disadvantage of the Kahn test, in my experience, has been the reading of the weakly positive sera. These are often difficult to interpret and on that account I have never felt secure in reporting a weakly positive Kahn unless I could check it with a complement-fixation test. On the whole, I believe that the Kahn test is a valuable addition to the diagnostic laboratory. It is an excellent check on the complement-fixation test because of its accuracy, simplicity in technic, rapidity in performance and inexpensiveness.

CLINICAL STUDY OF KAHN PRECIPITATION TEST AND KOLMER COMPLEMENT-FIXATION TEST*

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WASSERMANN, Neisser and Bruck,[1] introduced to the medical world the complement-fixation test for syphilis in 1906, and since that time it has undergone several modifications in the hope of making it more practicable and more sensitive with greater specificity; but in all of its modifications the fundamentals of the test are the same. The disadvantages of the complement-fixation test are well known. The number of biologic reagents that enter into the test increase the source of error. The most important ingredient, complement, is

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namely, Kolmer's standardized technic, which has been repeatedly shown to be practically specific. The sera obtained were tested by Miss Yagle in Dr. Kolmer's Laboratory, using his technic; and a portion of each serum was tested by me independently, using the Kahn technic. In order to be unbiased in my opinion, I read and recorded my findings before knowing the results of the tests performed by Miss Yagle.

EQUIPMENT

Glassware.—The test tubes for making the standard antigen were of standard size, 5.5 cm. in length and 1.5 cm. in width. The tubes for conducting the test were 7.5 cm. in length and 1 cm. in diameter. All test tubes were perfectly clean and free from acid and alkali and sterilized in hot air sterilizer. The pipettes were chemically clean and sterile. Five c.c. pipettes graduated to 0.1 c.c., one c.c. pipettes graduated to 0.01 c.c. and calibrated to the tip, and 0.2 c.c. graduated to 0.001 c.c. quantities were used.

Antigen.—The antigen used in my series of tests was furnished by Dr. Kahn, ready for dilution and sent to me at about one month intervals. The titration was usually 1 c.c. standard antigen plus 1 c.c., 1.1 c.c. or 1.2 c.c. physiologic salt solution.

Physiologic Salt Solution.—This was prepared by adding 8.5 grams of chemically pure sodium chlorid in 1000 c.c. of freshly distilled water in a chemically clean and dry flask, filtering and sterilizing in an Arnold sterilizer for one hour.

Technic.—The technic employed in conducting the tests was that advised by Kahn, with one exception. In performing his tests the sera were inactivated for thirty minutes at 56°. Kolmer[21] in his modification of the complement-fixation test found that heating the serum for thirty minutes at 56° results in the destruction of a portion of syphilis "reagin," and that heating for fifteen minutes at 55° is sufficient and results in less destruction of "reagin." As my series of cases were compared with Kolmer's modification of the complement-fixation test, his method of inactivation of sera was carried out; the tests were inactivated for fifteen minutes at 55° and were then put in the water-bath for fifteen minutes before adding 0.5 of physiologic salt solution.

Serum.—Specimens of blood were drawn and allowed to stand overnight. The following morning the sera were separated from the clots and made free from red cells, fibrin and other particles which would interfere with accurate readings. They were prepared as for the complement-fixation test (Kolmer) and inactivated for fifteen minutes at 55° C. as above noted. Each serum was then divided, a part being used for the complement-fixation test and a part for the precipitation test.

Antigen Dilution.—One c.c. of standard antigen was placed in a standard mixing tube, and approximately the same amount of physiologic salt solution as determined by titration was placed in a second standard mixing tube. The salt solution was quickly poured into the antigen, and without waiting for it to drain it was poured back and forth for six or eight times to insure thorough mixing. This antigen was allowed to stand for a few minutes before using, but was used in test within a half hour.

Conducting of the Test.—Three test tubes were employed for each serum tested. In the first tube was placed 0.05 c.c. antigen dilution, in the second tube 0.025 c.c., and in the third 0 0125 c.c. The antigen dilution was delivered to the bottom of each tube. Then to the antigen in each tube was added 0.15 c.c. of serum to be tested. After the serum was added, the test was shaken for two or three minutes to insure thorough mixing, and incubated in the water-bath at 38° for fifteen minutes. Five-tenths c.c. of physiologic salt solution was then added and the reading made. This was done before a window with dark background. The strongly positives were easily read, the weakly positives and doubtful reactions were read by slanting the tubes and looking for precipitation in the thin layer of fluid. After this reading was made the test was placed in the ice box overnight, and final reading made the following morning. Ice box incubation increased the degree of precipitation in the weaker reactions.

Method of Control.—Each test had the following controls: (1) Antigen. (2) Known positive serum. (3) Known negative serum. Each control contained the same amount of antigen dilution and serum as the test, while the antigen contained only the normal salt solution.

TABULATION OF CASES

As all of my series of cases except four were old untreated cases, I have tabulated the results merely as positive and negative, instead of giving the degree of positivity. In this type of case, as we are not using the test as a guide for treatment, we are not as much interested to know the degree of positivity of serum reaction.

LATENT SYPHILIS

NO. OF SERA	KAHN TEST	COMPLEMENT FIXATION	AGREEMENT	DIVERGENCE
67	66 positive	64 positive	63	4

Divergence—4 cases

Case No.	Kahn Test	Complement Fixation
1. (C.W.)	Positive	Negative
2. (J.S.)	Positive	Negative
3. (J.K.)	Positive	Negative
4. (G.D.)	Negative	Positive

The histories and clinical findings of these latent cases were rather indefinite, the majority of them being referred from other departments to Schamberg's Clinic for complement-fixation test. A few gave a history of a primary lesion; others denied all knowledge of infection. In the series of 67 cases there was disagreement in four cases. Three gave a positive Kahn test and a negative complement-fixation test, and one gave a negative Kahn and a positive complement-fixation test. It will be of interest to note that five cases out of sixty-seven cases of latent syphilis gave a negative complement-fixation test until the natural antisheep hemolysin was absorbed. After this they were positive. Also one case gave a positive Kahn and a negative complement-fixation test, but after four weeks of treatment the complement-fixation test was strongly positive. There is the possibility of missing a small percentage of positive cases unless the antisheep hemolysin is absorbed routinely.

Case Histories and Clinical Findings in four cases showing divergence of precipitin and complement-fixation tests follow:

CASE 1.—C. W., a man, aged fifty-four years, came to the Clinic October 28, 1918, with the following history: "Had a primary lesion two and one-half years ago, duration two or three months. Rash appeared and has been appearing and disappearing for the last two years. Has sore mouth, tongue beefy red, glazed, shows milky patches and is slightly lobulated. His wife has had three children, all of whom are living. She had one miscarriage eighteen years previously." The blood was taken and the Wassermann found to be strongly positive. The patient received ten weekly injections of arsenobenzol over a period of two months, with the Wassermann, showing strongly positive. The patient disappeared from the Clinic at the end of this time and did not report until January, 1925. Two blood tests taken then which were positive to the Kahn test and negative to the complement-fixation test.

Physical Examination at this time showed the following: Glossitis and lingual sclerosis; enlargement of the liver with nodular edges. The Department of Neurology reported the following: Pupils regular, react slightly to light and in accommodation; somewhat myotic, ocular movements normal; cranial nerves normal, reflexes all normal. Their conclusion is "The symptoms of neurosyphilis not very definite. Pupils are rather rigid. Slight tremor of facial muscles, failure of memory and fatigability. Might indicate a pre-paretic state. Whether or not I think would depend somewhat on serological test."

Spinal fluid examination showed the following: Complement-fixation test, negative, colloidal gold curve 1123430000, cells—17 per c.mm., globulin not increased.

CASE 2.—J. S., a man, aged thirty-nine years, came to the Medical Clinic January 1, 1925, complaining of a "heavy feeling" in the epigastric region. Physical findings: No palpable lymph nodes; bone and joint findings normal; patellar reflex active; pupils unequal and irregular, reaction to light sluggish, to accommodation normal; mouth and pharynx negative; lungs negative; heart rhythm irregular with dropped beat, systolic murmur at apex; liver normal.

The patient's wife is living and well. Three children are living and well. The last pregnancy resulted in a miscarriage at four months. The patient gave a history of being treated for syphilis three years ago by his private physician, and was told that he did not need any more treatment. His diagnosis in the Medical Clinic was "chronic myocarditis." He was referred to Dr. Schamberg's Clinic for a complement-fixation test. Two tests were made at about two week intervals and both were negative. Two Kahn tests were made on the same sera and both were positive. The patient left the city before a spinal puncture could be made. However, the history of treatment for syphilis, together with the unequal and irregular pupils and sluggish reaction to light, are very suggestive.

CASE 3.—J. K., private patient of Dr. Jay F. Schamberg. The patient, a man aged forty years, was first seen in December, 1912, with a history of having had a syphilitic infection three years before. He had a negative Wassermann at the time of his first visit to Dr. Schamberg, but owing to the inadequacy of his earlier treatment, he was given a course of arsphenamin injections. The patient resided in another city and his visits were rather irregular. Although free of symptoms, his Wassermann was positive in March, 1913. Another course of arsenical injections was given, followed by mercury. Two months later the Wassermann was still positive. Mercurial injections were then administered. In September, 1913, a quantitative test gave a four-plus reaction with four different antigens. Further treatment was given and in April, 1914, it was three-plus. Four months later it was two-plus. In January, 1915, the complement-fixation test was as follows: Cholesterinized beef heart four-plus, alcohol extract syphilitic liver four-plus. The patient was further treated, but at irregular intervals. Nine years then elapsed before the next visit in December, 1924, when the patient came to request an opinion as to eligibility for marriage. He had been free of all symptoms for over ten years and was in excellent health. His Wassermann by the Kolmer method was completely negative, but it was one-plus positive by the Kahn test. Owing to this, a second Kolmer test was performed two weeks later,

with again a negative outcome. Spinal puncture was performed and the four usual tests made, which were all negative. Permission for marriage was given.

It is impossible in this case to state whether the Kahn test was more sensitive than the Kolmer, or whether it gave a false positive.

CASE 4, G. D., a woman, aged thirty-four years, had a blood test taken by her private physician and this was found to be positive. She was referred to Dr. Schamberg's Clinic for antisyphilitic treatment. On examination nothing of importance was found. A complement-fixation test on the blood serum was positive, and a Kahn test on the same serum was negative. The complement-fixation test and the Kahn test were done on four consecutive days, with the complement-fixation test positive and the Kahn test negative. After three months treatment with neoarsphenamin another Kahn test was performed, again with negative result. The complement-fixation test during the three months treatment has been positive.

No theory is offered as to the reason for the negative Kahn test in this case.

TERTIARY SYPHILIS

NO. OF SERA	KAHN TEST	KOLMER COMPLEMENT FIXATION TEST	AGREEMENT	DIVERGENCE
34	34 Positive	34 Positive	34	0

NEUROSYPHILIS

NO. OF SERA	KAHN TEST	KOLMER COMPLEMENT FIXATION TEST	AGREEMENT	DIVERGENCE
6	6 Positive	5 Positive	5	1

Divergence 1 case

CASE NO.	KAHN TEST	KOLMER COMPLEMENT FIXATION TEST
Case 5. (M.M.)	Positive	Negative

CASE 5.—M. M., a man, aged fifty-one years, came to the Medical Clinic complaining of stiffness of the joints and aches and pains all over the body for the last six months. He has vertigo and staggering at times. On physical examination there are no palpable lymph nodes, the bones and joints are normal, the patellar reflexes are present when reinforced.

Eyes.—The right pupil is dilated, due to an injury. The left eye will not react to light or in accommodation. The heart and blood vessels are normal.

The patient was referred to Dr. Schamberg's Clinic for a complement-fixation test. The complement-fixation test was negative, and the Kahn test positive. Both tests were repeated one week later with the same results The antisheep hemolysin was absorbed and the complement-fixation test repeated, again with negative results.

Spinal puncture was done and the following tests made and recorded: Complement-fixation test, strongly positive; colloidal gold gave a luetic curve, cell 75, and increased protein.

In this case the Kahn test appeared to be more sensitive than the Kolmer test. This case and the preceding one illustrate the importance of making both tests.

CONGENITAL SYPHILIS

NO. OF SERA	KAHN TEST	KOLMER COMPLEMENT FIXATION TEST	AGREEMENT	DIVERGENCE
5	5 positive	5 posiitve	5	0

SUMMARY

In the clinical study of 110 cases a Kahn precipitation test and a Kolmer complement-fixation test were run on the same sera. The precipitation test was done by the method advised by Kahn, with the exceptions that inactivation was

carried on only fifteen minutes, and that the readings were checked the following morning after ice box incubation. In this way it was found that the doubtful and weaker reactions became more distinct.

In tests done on 110 cases, a remarkable concordance of results was found in 105, or 95.45 per cent. This indicates a high degree of sensitivity and specificity in both tests. In the five cases that disagreed, four are known to have had syphilis and the other case is clinically suggestive. Three of the cases that are known to have been syphilitic gave a positive Kahn and a negative complement-fixation test with blood sera. One case gave a repeated negative Kahn test in the face of a persistently positive complement-fixation test. Both tests were repeated after three months of neoarsphenamine treatment, and the same discrepancy again noted. No explanation is offered for this divergence, and it will remain for future workers to ascertain whether such false negatives occur with the Kahn test. The case which gave a history of treatment and presented suggestive clinical findings of syphilis gave two positive Kahn tests and two negative complement-fixation tests on two different sera. Out of 110 cases studied, it was found that five gave negative complement-fixation tests until the natural antisheep hemolysin was removed. One case gave a positive Kahn and a negative complement-fixation test and after four weeks of neoarsphenamine treatment the complement-fixation test was reversed to strongly positive.

In this series of cases there is no convincing evidence that a positive occurred with the Kahn or the Kolmer test which was inconsistent with clinical or other serologic findings. In Case 3 no final judgment is possible as to the positive Kahn test.

The present study shows a remarkable degree of harmony between the outcome of the Kahn precipitation test and the Kolmer complement-fixation test, and indicates the high degree of sensitivity and specificity common to both. It may not be essential to carry out both tests routinely, but in doubtful cases both should be performed as they shed a useful complementary light upon each other.

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THE NEW MODIFICATION OF THE MEINICKE TEST*

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SINCE the introduction of the Wassermann test and especially during the World War, many laboratory workers have tried to find a serodiagnosis test for syphilis, which will combine a specific reaction with simple working methods that will not require several unstable and expensive reagents. The nature of the Wassermann test was originally explained by Ehrlich's side chain theory until it became known that antigens prepared from normal extracts gave practically the same result as those made up from syphilitic livers, which were difficult to obtain for the ever-increasing demands. After that research took a different direction. Many new theories sprang up, numerous modifications of the Wassermann test were employed, new tests recommended, of which most of them soon fell into disuse. In recent years a new kind of test, the floccule formation test, has become known, has been tried by many and has stood the proof of time. At the meeting of the "League of Nations" in Switzerland in 1924, the floccule formation test, of which the Sachs-Georgi and the Meinicke are the best known, was cited as a reliable test in syphilis, giving the same reaction as the Wassermann and being especially useful where the latter is indefinite.

Many authors have endorsed the floccule formation test. A. L. Urquart in the Lancet recommends the Sachs-Georgi test on account of its reliability and simplicity. Levinson and Petersen have found the Sachs-Georgi test to be even more sensitive than the Wassermann in certain cases of neurosyphilis. Much has been written with regard to the true nature of the Wassermann, floccule formation and other immunity tests. Kolmer in his article in "Infection, Immunity and Serum Therapy," 1923, p. 523, expressed his belief that the Wassermann and the floccule formation tests have the same fundamental mechanism. In the Wassermann test there is also the formation of floccules which have been demonstrated by means of the ultramicroscope Both kinds of tests depend upon a specific antibody, just like agglutination and precipitin reactions. Wassermann to the last maintained that a true antibody is responsible for the complement fixation in syphilis. Meinicke explains the floccule formation by a

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special affinity of syphilitic sera for lipoidal extracts; this may be a true chemical change or an absorption. He considers the floccule formation to be a true immunity reaction, as the complement-fixation, agglutination and precipitin tests. Epstein and Paul consider the Wassermann and Meinicke reactions to be due to a specific protective colloid, which preserves the stability of the globulin molecule in normal sera and is absent in syphilis. H. G. Wells in his recently published book, "The Chemical Aspects of Immunity," expresses the opinion that at present we can say nothing more definite than that the proportion and character of serum globulins and lipoidal colloid suspensions are altered in syphilis in some unknown way, so that their stability is greatly reduced and hence there is a tendency to flocculation and to absorption of other colloids, including among them the serum colloids, which exhibit the function of complement. There is no convincing evidence that a true antibody produces the change in the behavior of the globulins.

Although the true nature of the floccule formation tests is not as yet fully understood, they doubtless are of great value. Several months ago we used a series of 500 specimens to check up on the Sachs-Georgi test in connection with the Wassermann. We proceeded to make up alcoholic extracts of dried beef heart muscle fortified with cholesterin, according to the Sachs-Georgi directions. Each new batch of antigen was titrated, using increasing amounts of cholesterin with several known positive and a few negative specimens until the proper amount for the most definite floccule formation was ascertained. The amount of cholesterin needed in the various new antigens was not at all constant, some extracts requiring more, others less for satisfactory results. A few antigens were found to be entirely ineffective and had to be discarded. All of the other antigens were rather unstable. For the test proper we mixed the inactivated blood sera with the extract, incubated for two hours and then made a preliminary reading. After that they were put back into the incubator until the final reading the next morning. The length of time required for the test proved to be the greatest difficulty in our work. Practically all of our specimens come from a distance and are from two to four days old when they reach us. Although we used a careful aseptic technic we got cloudiness in some specimens, which was due to bacterial growth. The results often were so indefinite as to make the reading very difficult.

In the fall of 1924 the author had a chance to become well acquainted with the Meinicke test, which is being used extensively in European laboratories. The Meinicke test is based upon the same principle as the Sachs-Georgi, the Kahn and other floccule formation tests, but in its present modification it is a step forward so far as simplicity in technic and a more definite reading are concerned. Meinicke uses horse heart muscle extract, without cholesterin, but with the addition of tulol-balsam. Most laboratories find it convenient to obtain the prepared extract from the Meinicke laboratories in Hagen-Westfalen, Germany, direct, since it is rather inexpensive, stable, and difficult to prepare. In the new modification test it is not necessary to wait for the formation of floccules since the production of an opacity in positive specimens is always the forerunner of the floccules and can be seen after keeping the test tubes at room temperature for one hour. The main advantage of the Meinicke test is that

specimens need not be inactivated, nor incubated and that it is completed within one hour, when a distinct reading can be made.

TECHNIC OF TEST FOR TEN SPECIMENS

Twenty small test tubes of about 4 c.c. capacity are set up in a double row. The front row is for control, the back row for the test proper, so that for each specimen there are two tubes. Of every specimen 0.2 c.c. of blood serum entirely free of cells is measured into the front and the same amount into the corresponding back row tube. All ten front row tubes receive one drop of pure formalin.

Dilution of extract: 1 c.c. of the Meinicke extract is measured into each of two 20 c.c. test tubes, which must be perfectly dry. Ten c.c. of a 3 per cent salt solution is pipetted into each of two other test tubes of even size. All four tubes are kept in a water-bath of a temperature not exceeding 45 and not falling below 40 degrees centigrade, for ten minutes. The warm salt solution is *mixed as quickly as physically possible with the warmed extract, at once drawn* up into a 10 c.c. pipette and 1 c.c. of the dilution added to each tube in the front and in the back rows. It is very important to mix quickly, as the suspension progressively becomes opaque, interfering with the reading later on. The control tubes in the front row and the negative tubes in the back row are entirely transparent, even though they have a slight milky appearance. The positive tubes have become opaque. This opaqueness can ONLY be seen by holding the tube up to the window in daylight. The meshes of the window screen or the wood work look hazy or cannot be seen at all when looking straight through the fluid of the tube. Out of 1000 blood serum specimens which we tested by both the Wassermann and Meinicke methods, we got the following results:

Wassermann		*Meinicke*	
negative	811	negative	811
positive	114	positive	114
anticompl.	9	negative	9
antic.	13	positive	13
doubtful	12	positive	12
doubtful	7	negative	7
negative	11	doubtful	11
positive	8	doubtful	8
negative	9	positive	9
positive	6	negative	6

The above Meinicke findings were read some two hours before the Wassermann results were known.

The Meinicke test is especially valuable in cases where the Wassermann is anticomplementary or doubtful. In our laboratory where most of the specimens are sent in from a distance we have found the Meinicke a great help when a specimen had become slightly hemolytic or anticomplementary during shipment. In those few specimens where Wassermann was distinctly contradictory to the Meinicke, we have asked for additional specimens. From our experience we can recommend the Meinicke test as a check on the complement-fixation test; it is simple, requires no special apparatus nor many reagents and gives a very distinct clear-cut reading.

MICROREACTION

In his most recent publication Meinicke describes the microreaction which can be carried out by the hanging drop method and requires only a platinum loop full of clear serum for the test. The fact that the specimens do not need to be inactivated has made this method practicable. The specimen can be obtained from the ear, one drop of blood being drawn up into a capillary tube of 10 cm. in length and 1 mm. in width. One end of the tube is sealed if the examination is to be made in the same place, otherwise both ends need to be sealed. After coagulation has taken place this tube is centrifuged and that part of the tube containing the clear serum is separated from the rest by means of a glass file and is then ready for use.

A cover-glass preparation is made, using five loopfuls of the diluted extract to one loopful of the patient's serum. All six drops are thoroughly mixed and the hanging drop made in the usual way. The slide is kept in the dark at room temperature for one hour, when the reading can be made by means of the microscope, using the high dry lens.

In the negative specimen the field looks almost empty, only here and there some dancing points can be seen. In the positive specimen a definite clumping with lack of motion is readily found, giving a similar picture to a positive Widal.

We have not as yet had a chance to try out this method on a large scale but consider it worthy of careful investigation.

DISCUSSION OF PAPERS BY SANFORD, CASSELMAN, KILDUFFE, OWEN, GIORDANO, KELLY, SAUNDERS

Dr. M. E. Marten.—Evidence seems to be accumulating that lipoids and cholesterin are tied up with immunity. I have found that in a large number of bloods which contain large amounts of fat, in conditions where a cholesteremia is present, e.g., syphilis, advanced nephritis, placental bloods, inhibition of hemolysis is common. Dr. H. N. Cooper's paper was very interesting and seems also to bear out some of these points. I have performed a series of experiments selecting saponin as the hemolytic agent, which I titrated against a unit volume of sheep's blood. Having obtained my hemolytic unit, I added cholesterin to a point where total inhibition or protection to lysis (for one unit volume of blood) occurred. This amount, I called the protective unit against one unit of saponin. I took a large series of Four-Plus Wassermann sera and found that far smaller amounts of protective agents (cholesterin) was necessary to prevent hemolysis. Indeed, in some reactions, practically no cholesterin was required, inhibition of hemolysis taking place even in the presence of one unit of saponin. The average amount of cholesterin required to protect against lysis in Four-Plus sera, with one unit of saponin, has not as yet been definitely determined by me; but if this should prove to be an average fixed amount, that is, a fixed or constant index, which I believe is very probable, no immune hemolytic sera or guinea pig complement would be necessary and a means for testing positive luetic sera could be accomplished, without the use of variable reagents as ambocepter and complement.

Dr. A. J. Casselman.—The reason anticomplementary spinal fluid is not anticomplementary when mixed with serum is that normal serum has a protective action on complement. As a rule there is less fixation of complement by antigen in the presence of normal serum than there is without the serum. This fixation to which I am referring is a nonspecific fixation due to the anticomplementary properties of antigen. I have a record of some experiments which I made during the war in Paris on increasing the sensitivity of antigen. Ice-box fixation could not always be used because of occasional difficulties in obtaining sufficient ice. I found the most sensitive method to be the use of a highly anti-

complementary antigen, but I always mixed the antigen and serum and allowed it to stand for fifteen minutes, before adding the complement. Some antigens were used in such concentration that without any serum they would cause complete fixation of complement, whereas in the presence of normal serum there was no fixation of complement. Very weak positives were picked up by this method which apparently did not give false positives. Even slightly anticomplementary serums protected the complement against the anticomplementary action of the antigen to a certain extent, as these two anticomplementary factors each acted differently and there was not any marked summation of their anticomplementary properties. This method of increasing the sensitivity of the Wassermann reaction was not considered suitable for routine work in the hands of technicians, but serves to illustrate the great protective action of most normal serums on complement, when the serum is mixed with antigen and allowed to stand before adding the complement.

Dr. Henry Steuart—It has been a great gratification to me to note the eminently fair manner in which you gentlemen have treated the Kahn test I believe it is of value, especially in the small hospital and as a check on the Wassermann. For the obvious reason of the disproportion between the expense of time and money, and the volume of work, I do not do the Wassermann, but I paralled all that I send away with the Kahn. In several instances the question raised by a positive Kahn has resulted in the correction of a negative Wassermann report. This is not a reflection on the Wassermann or the men who did it. It is simply an exposition of the fact that all the disadvantages do not inherently lie in the small laboratory—for every individual added to the personnel increases the element of human liability to error

For the intrinsic merit of the test, let me give you one case.

A patient with supposed carcinoma of the stomach was admitted to the house. Our diagnosis was neoplasm of the esophagus or mediastinum He had lost one hundred pounds in less than a year. I was not able to pass the stomach tube. The dysphagia was frightful —it took him half an hour to get down his dose of barium. On the strength of a positive Kahn I began salvarsan and five days from that time he was swallowing in comparative comfort, and in a week the dysphagia had disappeared The Wassermann report came negative; notwithstanding that, I continued the salvarsan for some weeks. There was no further improvement after the second dose, he resumed his loss of weight, which had been temporarily checked He passed out of our hands, and died in about three months, but the dysphagia did not return

If there were no other consideration, I feel that the euthanasia of that one man abundantly repaid me for all the time I have spent on the Kahn test.

Dr. Herman Spitz—I have attempted the Kahn test with four specimens of cholesterin antigen sent to me by Dr. Kahn. Due to my inexperience in reading flocculation and precipitation reactions I was unable at that time to get any satisfactory results and discontinued the use of the Kahn test. One of our colleagues in Nashville is using the Meinicke test as a check on the Wassermann. He is not prepared at this time to publish the results but we have found a peculiar factor in its use with spinal fluids. Spinal fluids showing positive Wassermann were consistently negative with the Meinicke test. We accidently discovered that adding a small amount of fresh complement to the test gave a uniform positive reaction with positive fluids. This fact may have been noticed by others, I am not very familiar with the literature on this subject and am therefore not claiming anything original in this observation.

Dr. H. J. Nichols.—I was interested in Dr. Sanford's paper as I did not know before that the anticomplementary reaction had any significance, clinically. There must be several different kinds of anticomplementary sera. One of our cases, with a negative Wassermann reaction, developed an anticomplementary reaction each time he took a course of arsphenamine.

It is encouraging that there is such a close parallelism between the Kahn and Wassermann reactions. In the cases in which there is a discrepancy, it would seem as though an analysis would tell us something about that. We have had a little experience along these

made in experimental animals. In the remaining number either a fall or a fall followed by a rise was observed.

This work was undertaken to determine if these substances possess blood pressure lowering properties when given by mouth in clinical cases of arterial hypertension in doses designated by the manufacturers.

METHOD

The method of taking the blood pressure was the same as that employed in previous investigations[2, 4] and requires little further description here. The maximum pressures were determined with a stethoscope by the Korotkoff sounds.[5] The fourth sound was taken as the diastolic pressure. The patients were seated near a desk in a quiet room. The left arm, from which the blood pressure readings were made, rested comfortably upon the desk. Blood pressure readings were taken within two minutes after the patients had sat down and thereafter at ten-minute intervals for twenty or more minutes. These pressures were then averaged to give the results presented in the table.

The drugs were administered in tablet form. Either sodium benzyl succinate 5 grains, or sodium dibenzyl phosphate 7½ grains were given three or four times a day.

RESULTS

The results obtained upon oral administration of sodium dibenzyl phosphate and sodium benzyl succinate in clinical cases of arterial hypertension were unsatisfactory. (See table.) The observations recorded in this table were made during May and June and September and October, 1924, in the outpatient department of Washington University Medical School. The patients were kept on the same diet and the administration of fifteen drops of a saturated aqueous solution of potassium iodide three times a day after meals was continued throughout.

Upon comparing the blood pressures before and during treatment it will be seen that it remained the same or was slightly elevated by the drug. The final average of the 18 averages made from the results on the 13 patients before the treatment was systolic blood pressure 182 and diastolic pressure 100 mm. of mercury. After seven days medication this average pressure was 186 systolic and 102 diastolic. As will be seen the pressure remained practically the same and is I believe within the normal variation and error. Thirteen observations were made fourteen days after beginning medication in eleven clinical cases. The average blood pressure in these cases before medication was 179 systolic and 99 diastolic, while after having taken the drug for fourteen days they were 188 and 109 respectively.

The pulse appeared to be unchanged in most cases, in one however there appared to be a marked acceleration similar to that found in our experiments upon animals. In this case the blood pressure and pulse changed as follows:

	SYSTOLIC BLOOD PRESSURE IN MM. HG.	DIASTOLIC BLOOD PRESSURE IN MM. HG.	PULSE PER MINUTE
Before treatment	174	81	96
During treatment			
7th day	176	81	100
14th day	199	89	116
After cessation of treatment for 10 days	183	77	88

TABLE OF RESULTS

A table showing the effect of sodium benzyl succinate "Benzycin" and sodium dibenzyl phosphate "Benzyphos" upon the blood pressure in clinical cases with arterial hypertension when taken by mouth. The case numbers here correspond to the numbers used in a previous communication (4 table I). All patients gave a negative blood Wassermann except XXIV which was 4+. Only the average of the three ten-minute readings is here presented. All observations were made on the seventh and fourteenth days after the beginning of treatment except as follows: a—third day; b—fifth day, c—tenth day; d—twelfth day. Sodium benzyl succinate* was taken in 5 grain tablets and sodium dibenzyl phosphate in 7½ grain tablets three and four times a day.

CASE	BLOOD PRESSURE IN MM. MERCURY BEFORE TREATMENT	BLOOD PRESSURE SEVEN DAYS AFTER BEGINNING TREATMENT	BLOOD PRESSURE FOURTEEN DAYS AFTER BEGINNING TREATMENT
II	161– 91	*178– 98	163– 93
III	237– 98	247–103-b	232– 99-d
VIII	186– 98	174– 94-b	
	174– 94	*171– 95	
	169–100	193–103-a	188–102
IX	165–118	*169–119	157–118
XIII	215–104	205–106	193– 94
	217–105	*216–107	
	212–109	*236–119	233–118
XIX	191–109	*208–114	220–120-c
	173–106	189–114	190–116
XXI	142– 77	*157– 81	165– 85
XXIV	206–115	*200–108	
XXV	174– 81	*178– 81	199– 89
XXVI	179–107	168–107	178–105
D. A. F. C. 39	169–103	*174–107	
C. C. F. W. 38	174–106	164–111	182–101
A. J. M. W. 66	132– 75	*127– 73	144– 81
Average	182–100	186–102	
Average	179– 99		188–109

The temperature was found to be the same at each of these determinations. Whether the increase in pressure caused the increased pulse rate in this case or whether the increased heart rate caused an increased volume output by the heart per unit of time thus causing the rise in pressure cannot be stated.

As our previous observations upon animals[3] showed that sodium benzyl succinate caused a rise in blood pressure in 67 per cent of the injections and sodium dibenzyl phosphate in 35 per cent of the cases, that the heart is slowed in many cases, in others markedly accelerated, and that sodium dibenzyl phosphate causes a precipitate in the presence of calcium salts it would seem inadvisable to use these drugs in arterial hypertension. Intravenous administration of the latter drug would certainly not be wise.

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INTESTINAL OBSTRUCTION AND PERNICIOUS ANEMIA: REPORT OF A CASE*

By Russell L. Haden, M.D., Kansas City, Mo.

TRUE pernicious anemia is a clinical entity seemingly due to a specific toxic agent. The nature and mode of origin of the toxin are unknown. Clinical evidence strongly suggests that it arises within the gastrointestinal tract. Glossitis, diarrhea, achlorhydria, and atrophy of the gastric mucosa are quite constant findings at some stage of the disease. A diagnosis of pernicious anemia is always questionable if free hydrochloric acid is found on gastric analysis. Hartman[1] has reported two cases in which pernicious anemia followed gastrectomy. Occasionally, cases of malignant disease of the gastrointestinal tract, usually of the stomach or colon, are encountered, in which an anemia of the pernicious type occurs. It is also well known that a blood picture simulating pernicious anemia may be due to infestation of the intestinal tract by certain parasites, especially the fish tapeworm, Dibothryocephalus latus. It seems possible that in all such cases there is a toxin present, identical with that occurring in idiopathic pernicious anemia. This supposition has stimulated interest in cases of primary anemia of known etiology, in the hope that information may be gained concerning the origin and nature of the toxin responsible for idiopathic pernicious anemia.

Further evidence in favor of the gastrointestinal origin of the hemolytic toxin is found in the occasional occurrence of the clinical picture of pernicious anemia with chronic intestinal obstruction. The association of these two conditions seems more than a coincidence. The first case was reported by Knud Faber,[2] in 1895. Meulengracht[3] has recently reviewed all previously reported cases and added several which have come under his observation. Faber suggested that hemotoxic substances must be absorbed from the intestine as the result of the stasis.

On the supposition that there is some abnormal absorption as a result of stasis in the intestine, Seyderhelm[4] did an enterostomy in ten cases of pernicious anemia. He noted a rapid improvement in the anemia after operation, with a relapse after the enterostomy opening was closed. Dixon[5] has recently reported like results in a series of cases in which similar operative treatment was given. Seyderhelm[6] was able also to produce, experimentally in dogs, a macrocytic anemia, by stenosing the small intestine near the ileocecal valve.

The cases of pernicious anemia and intestinal obstruction reported by Meulengracht[3] are briefly abstracted below:

Case 1.—A woman, age seventy-five years, had had diarrhea and vomiting for several years, and a marked anemia for some time. The red corpuscles were 1.40 million and the hemoglobin 40 per cent. The red cells showed poikilocytosis and macrocytosis. Free hydro-

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chloric acid was absent on gastric analysis. The autopsy revealed an erythroblastic red marrow, and several benign strictures in the lower part of the jejunum and ileum.

CASE 2.—A woman, age twenty seven years, complained of an intestinal disturbance for one year with irregular stools and slight vomiting. There was a progressive anemia, with a febrile period before death. The red corpuscles numbered 0.60 million and showed poikilocytosis with many macrocytes. The hemoglobin was 20 per cent. At autopsy two benign strictures 2 and 3.5 meters below the pylorus with a dilatation above the obstruction were found.

CASE 3.—A woman, aged forty-nine, had an anemia for two years. During that time there were dyspeptic symptoms, constipation and meteorism, with fever before death. The hemoglobin was 20 per cent. The red count was 0.48 million with a striking macrocytosis. There was no leucocytosis. Fourteen strictures of a fibrous nature were found in the ileum at autopsy.

CASE 4.—A male, aged twenty-eight years, complained for several years of pain in the abdomen and vomiting. The hemoglobin was 20 per cent, the red count 1.0 million and the white corpuscles 2,000. Macrocytosis was marked. The patient died following an exploratory operation. At autopsy a fibrous stricture in the jejunum, seven feet from the duodenum, was found. There was red bone marrow in the long bones. The liver, spleen, and kidneys showed an increased iron content.

CASE 5.—A woman, aged thirty-eight years, complained of swelling of the feet and legs, vomiting, and weakness The hemoglobin was 50 per cent and the red cells 2.70 million. There was marked poikilocytosis, anisocytosis, polychromasia, and many nucleated forms. There was no free HCl in the gastric contents. The patient had slight fever, alternating constipation and diarrhea, increasing edema and anemia. At autopsy, there was a fibrous stricture in the upper part of the colon through which the finger could not be passed.

CASE 6.—A woman, age fifty-seven years, was examined in 1914, and found to have a hemoglobin of 73 per cent. She had a tendency to diarrhea, and a paresthesia of the foot. Two years later the hemoglobin was 39 per cent, and the red corpuscles 5,000. The red cells showed definite poikilocytosis, anisocytosis and macrocytosis. Gastric analysis showed no free HCl. Following this, there was some improvement in the blood condition with a subsequent relapse and death nine months later. The patient had a glossitis. The blood plasma contained bilirubin. At autopsy, there was atrophy of the papilla of the tongue, and red bone marrow in the long bones. One meter above the ileocecal valve, there was a benign fibrous stricture through which the finger could be scarcely passed.

CASE 7.—A woman, at the age of fifty-eight years, was admitted to the hospital for abdominal pain and vomiting. The gastric analysis showed the presence of free HCl. At operation a benign stricture of the intestine was found and resected. Six years later she returned, complaining of colicliko pains and a diarrhea of two years duration. A gastric analysis showed 60 acidity per cent of free HCl. The hemoglobin was 49 per cent, the red count 2.42 million and the leucocytes 4,000. Two months later the hemoglobin was 52 per cent, the red corpuscles 1.20 million, and the white corpuscles 3,400. The red cells showed anisocytosis and poikilocytosis. At operation, three benign strictures in the upper part of the ileum over a space of 0.5 meter were found. The patient died following the operation. There were abundant bacteria above the obstruction at a level at which bacteria are usually absent.

The essential facts in the case I have to report are as follows:

History.—L. H. B., a housewife, aged sixty-four, was first seen December 6, 1923, complaining of pain in the right side of the abdomen. The family history was unimportant. She had always been well except for typhoid fever and malaria many years before. The patient dated her present illness from an attack of influenza, in 1920. Following this, she began to have anorexia, eructation of gas, qualitative food distress, and constipation. She had had some attacks of pain in the gall bladder region but no colic. The attacks lasted

from one to two weeks and at times required hypodermics for relief. She stated that she had been losing weight for two weeks.

Examination.—The patient showed no evidence of marked loss of weight. There was no jaundice. In the region of the gall bladder, there was a mass extending a hands breadth below the costal margin. The mass was firm but not nodular, and quite tender on palpation. No evidence of involvement of the nervous system was found. There was some atrophy of the tongue. The examination was otherwise negative.

The fluoroscopic examination showed the stomach slightly enlarged, with some hyperperistalsis. There was no filling defect. The duodenal cap was fairly well visualized and appeared large, although no deformity was found. A radiograph of the gall bladder area was negative for calculi.

The urine examination showed only a trace of albumin. The gastric analysis revealed no free HCl and the total acidity was low. The blood examination showed 1,872,000 red corpuscles, and 4,650 white corpuscles, with a normal differential count. The hemoglobin was 51 per cent. The color index and volume index were 1.36. The red cells showed macrocytosis but very little anisocytosis or poikilocytosis. The platelets were diminished. No nucleated red cells were found.

Further Course.—The patient was given Fowler's solution and dilute HCl, and began to improve. Two weeks later she was again seen. She stated that she had been markedly constipated for one week. During that time she had also had cramplike pains in the abdomen with nausea and vomiting, and had developed a glossitis. She was admitted to the hospital December 22, 1923. For the next week she had fever, the temperature rising over 102 degrees. She was delirious at times and had involuntary bowel movements. The abdominal pain, and the vomiting continued. She was given large quantities of 2 per cent sodium chloride solution, subcutaneously. The temperature gradually fell to normal, although the other symptoms were little changed. January 15, she again began to have a slight rise in temperature, a cough with tenacious sputum and signs of consolidation of a portion of the right lower lobe. The sputum contained large numbers of Monilia. On giving potassium iodide, the signs and symptoms of pulmonary infection rapidly cleared up. The patient gradually lost weight, became progressively worse, and died February 11, 1924. During the time the general symptoms were becoming worse, the blood continued to improve, as shown in Table I. The vomiting before death was fecal in character. The chloride content of the blood was quite low. This is typical of intestinal obstruction.[7] The possible relation of the chloride metabolism of pernicious anemia has been discussed elsewhere.[8]

Autopsy.—The postmortem examination was made soon after death. Only the important findings are reported.

The body was markedly emaciated. There was a slight icteric tint to the skin. The subcutaneous fat had a peculiar lemon yellow color. The lungs were entirely negative.

There was a mass of adhesions in the gall bladder region involving the hepatic flexure of the colon, the duodenum and the pancreas. The large intestine was markedly dilated along the ascending colon and cecum with a complete constriction of the remainder of the colon. In the neighborhood of the gall bladder there was a perforation through the cecum about two inches in diameter, rather sharply defined, and opening into the large cavity. Below the perforation, the ascending colon was markedly constricted so the lumen was almost obliterated. The middle portion of the duodenum was markedly constricted and opened into the same cavity. (Fig. 1.) On passing the finger up the duodenum from the lower end, it entered the cavity. The cavity when opened, measured about six inches in diameter and contained foul smelling thick exudate. The eroded remnant of the gall bladder projected into the cavity. There were calculi in the cystic and common bile ducts. The wall of the cavity passing into the liver consisted of a zone of brown friable cellular tissue, from one to three inches in thickness, extending into the liver substance. There were isolated nodules here and there in the liver. Underneath the cavity and extending under the pancreas there were several large irregular lobulated tumor masses, suggesting enlarged lymph glands. These extended between the hilum of the liver and the pancreas. A portion of the ileum was caught in the pelvis and was distended. The mucosa showed several ir-

regular and punched out ulcers. The liver tissue, away from the tumor tissue, showed fatty degeneration especially around the periphery of the lobules.

The bone marrow from the femur was removed and showed typical grayish pink cellular bone marrow with only a few islands of fatty tissue still persisting.

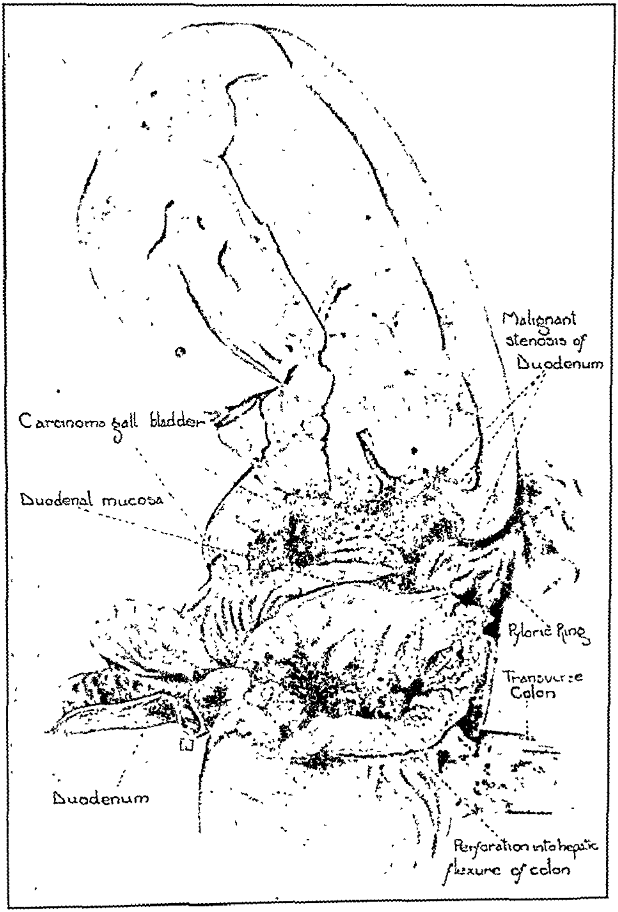

Fig. 1.

The sections showed an early pneumonic reaction in the lungs. There was an increase in fibrous tissue in the portal spaces of the liver with fatty change. Sections of the tumor showed epithelial architecture with marked tendency to mucoid degeneration. Mitotic figures were very numerous.

TABLE I

BLOOD FINDINGS DURING OBSERVATION

DATE	RED CORPUSCLES IN MILLIONS	WHITE CORPUSCLES	HEMOGLOBIN	COLOR INDEX	VOLUME INDEX	SATURATION INDEX	BLOOD CHLORIDES AS NaCl IN MG. PER 100 C.C.
Dec. 6, 1923	1.87	4650	51	1.36	1.36	1.00	550
Dec. 21, 1923	2.43	4000	53	1.09	1.22	0.90	400
Jan. 16, 1924	3.52						330
Jan. 25, 1924	3.97		80	1.00	1.01	0.99	370
Feb. 5, 1924	3.14	3800	62	1.00	0.98	1.02	405

The spleen showed a considerable increase in fibrous tissue throughout the pulp. No blood pigment was seen in the spleen. A small amount of blood pigment was seen in the central zone of the liver.

The sections of the bone marrow showed many foci of myeloid cells scattered throughout the fat tissue, presenting a picture fairly typical of a change of fatty bone marrow to red bone marrow.

The anatomic diagnosis was primary carcinoma of the gall bladder with metastasis to the regional lymph nodes and to the liver, and direct extension, with perforation, into the duodenum and the colon; malignant stricture of the hepatic flexure of the colon with dilatation of the ascending colon; partial obstruction of the duodenum, and myeloid hyperplasia of the bone marrow of the femur.

DISCUSSION

This is a case of clinically typical pernicious anemia occurring with a malignant tumor of the gall bladder and obstruction of the duodenum and colon. It is possible that the two conditions represent coincident diseases. It is most probable, however, that the toxic agent responsible for the anemia has arisen as a result of the stasis or of some alteration in function of the intestine, induced by the presence and perhaps pressure of the tumor. There is no doubt that a macrocytic anemia, typical clinically and at autopsy of pernicious anemia, may result from intestinal obstruction. This may be due to the development and absorption of hemotoxic substances within the lumen of the gut as suggested by Faber; or to some alteration in function, such as is responsible for the toxemia of intestinal obstruction. Cases of true primary macrocytic anemia are after all very rare with malignant tumors. These occur only with tumors involving the stomach and intestine. It is a question whether in such cases the toxic agent does not arise in the intestinal tract, probably as a result of the obstruction, rather than in the malignant tumor tissue.

The rapid improvement in this patient's blood condition under the treatment which benefits pernicious anemia so markedly, suggests that we are dealing here with the toxin responsible for the symptoms of idiopathic pernicious anemia. This improvement continued even with the increase in size of the primary tumor. One would not expect improvement to occur were the toxic agent arising in the tumor tissue.

The occurrence of such cases as this is further evidence of the intestinal origin of pernicious anemia.

SUMMARY AND CONCLUSIONS

Previously reported cases of pernicious anemia and chronic intestinal obstruction and the evidence suggesting a casual relation between the two conditions are reviewed.

A case of clinically typical pernicious anemia occurring with a malignant obstruction of the duodenum and colon is reported.

It is quite probable that the hemotoxic agent responsible in this case for the anemia is identical with that occurring in idiopathic pernicious anemia. The toxin possibly arises as a result of the stasis or disturbance of function within the intestine rather than from the malignant tumor tissue.

There was a marked improvement in the blood under treatment, although the tumor continued to increase in size.

The findings here are further evidence of the intestinal origin of pernicious anemia.

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ON THE USE OF RAW AND INACTIVATED SERUMS IN A FLOCCULATION TEST FOR SYPHILIS*

By C. B. McGlumphy, M.D., and W. W. Brandes, B.A., Chicago, Ill.

THE various precipitation tests for the serum diagnosis of syphilis are undergoing a thorough trial as to their practical value and doubtless the place which they are to occupy among laboratory methods will soon be definitely determined. Since the technic of the precipitation tests is such that they may be applied under circumstances that might prevent the application of the Wassermann reaction such tests may furnish valuable information to the clinician. Any modification which would further simplify or increase the sensitiveness of these reactions would be of value.

It may be mentioned that some workers would substitute certain precipitation tests for the Wassermann reaction since they believe that the precipitation methods, which are much simpler, are also more sensitive in detecting certain cases of syphilitic infection. Many who have made a comparative study of the two reactions advise the precipitation test as a check on the Wassermann test.

Epstein and Paul[1] reported a series of eleven thousand cases in which the Wassermann and Meinicke reactions were carried out simultaneously and con-

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cluded that the Meinicke reaction is more sensitive in the detection of lues in all stages, and that with the latter method false positive reactions do not occur as is the case with the Wassermann test. These workers believe that if a single laboratory test is to be used the Meinicke reaction is to be preferred to the Wassermann, but that the optimum serodiagnostic method is the application of both these reactions. A few cases in which the Wassermann and Meinicke tests gave different results came to autopsy and the gross and microscopic findings confirmed the result of the Meinicke test which was positive.

Weil and Keim[2] reported a series of cases which were tested with the Wassermann reaction and the Kahn precipitation test and came to the conclusion that the precipitation method, as checked by the clinical data, compared favorably with the Wassermann method in sensitiveness. The conclusions of these authors are cited as typical of the favorable reports on two methods which are used in many laboratories and which are based on the same general principle.

Weil and Keim heated the serums at 56° C. for one-half hour before mixing serum and antigen. Kahn[3] has recommended that the serums be heated for one-half hour at 56° C. for the purpose of removing inhibiting substances. Epstein and Paul[1] advise the use of unheated serum in the Meinicke test and state that a higher percentage of positive reactions are obtained than when the serums are heated at 56° C. for one-half hour.

In a recent paper by one of us a flocculation test for syphilis was described[4] and the results in a series of cases in which this method was used were tabulated. With this flocculation method less favorable results were obtained than with the Wassermann reaction. This was especially true of treated cases of syphilis which gave a three-plus or a two-plus Wassermann. However, a number of cases which clinically were nonluetic gave positive Wassermann and negative flocculation reactions. In this series inactivated serums were used.

For the purpose of studying the influence of heat on the flocculation reaction a small number of serums were tested before and after heating at 56° C. for one-half hour. Some of the tubes containing heated serums which gave a positive reaction showed the presence of small floccules and would be classed as weak reactions, whereas the corresponding tubes containing unheated serums showed the presence of large floccules and would be classed as strong reactions. In addition a small series of cases were studied in which the serums were heated a second time at 56° C. and the flocculation test then made in the usual manner. The interval between the two periods of heating was approximately four hours. The results in this series showed in a striking manner that two periods of heating at 56° C. weakened the reaction in a marked degree in a large proportion of the positive cases and it did not seem worth while to make further tests with serums which had been subjected to two periods of heating. Whether to use raw or inactivated serums in the flocculation test seemed to be a question of considerable importance because of the possibility of increasing the number of positive results.

The present paper deals primarily with the results obtained in a series of cases in which the flocculation test was made with serums before and after heating at 56° C. for one-half hour. That conditions would be the same for all

tests serum was removed before heating and kept under the same conditions, except for the period of inactivation, as the heated serums. A sufficient amount of the diluted alcoholic extract for both series of tests was prepared and added to both sets of serums after which they were incubated at 37° C. and readings made after eighteen hours and again after thirty-six hours. The method of preparing alcoholic extract of beef heart and performing the flocculation test has been described in a previous paper and will be outlined briefly.

PREPARATION OF ALCOHOLIC EXTRACT

Ten grams of dried and powdered beef heart extract are extracted with 90 c.c. of ether for twelve hours at room temperature. The supernatant ether is then poured off and the extraction repeated twice with fresh ether, the mixture being well shaken several times during each period of extraction. The last ether is poured off and the powder kept at 37° C. until no odor of ether can be detected after which 90 c.c. of 95 per cent alcohol are added to the container and extraction again made at 37° C. for forty-eight to seventy-two hours. The alcoholic solution is then filtered and without further preparation is ready for dilution and addition to the serums to be tested. In our experience such a procedure has always yielded a satisfactory extract.

FLOCCULATION TEST

For each test 0.1 c.c. of the alcoholic extract is thoroughly mixed with an equal amount of glycerol, 0.6 c.c. of 3 per cent sodium chloride solution added and the whole well shaken. This mixture is added to 0.2 to 0.3 c.c. of serum, the containing tubes well shaken and incubated for twenty-four to forty-eight hours at 37° C. Positive tests are recognized by the presence of white floccules of various dimensions. Negative tests show no change from the original appearance of the mixture of serum and diluted extract.

WASSERMANN METHOD

Acetone insoluble extracts of beef heart, one with and one without the addition of cholesterin, were used as antigens. Antigen, serum and complement were mixed and incubated for one-half hour at 37° C. Antisheep amboceptor and sheep cells were then added and incubation repeated for one-half hour and the results noted.

Since we were interested in studying the results of the flocculation test with raw and inactivated serums, clinical data and the results of the Wassermann reaction have been included only in those cases which showed different results before and after heating at 56° C. for one-half hour. The serums were obtained from the dispensary service of the Northwestern University Medical School in which the Wassermann test is carried out as a routine procedure. The Wassermann statistics and the clinical data were obtained from the dispensary records.

The results of the flocculation test in a series of 240 serums which were tested before and after inactivation are shown in Table I. This selected series includes all the positive cases observed during the examination of approximately two thousand serums.

LABORATORY METHODS

A SIMPLE METHOD FOR MEASURING SURFACE TENSION CHANGES OF PURE AND BIOLOGIC FLUIDS*

By Floyd De Eds, Ph.D., San Francisco, Cal.

THE measurement of surface tension of biologic fluids presents certain difficulties not encountered with pure liquids, such as water, benzene, alcohol, etc. Biologic fluids are complex colloidal mixtures and frequently contain substances capable of lowering surface tension. It is the latter factor especially which may be a source of error in surface tension measurements made by means of the stalagmometer. If, for example, the surface tension of blood serum, or of plasma, is determined by the stalagmometer, the constituents which lower the tension tend to concentrate in the surface of the drop as it forms, thus permitting the formation of a greater number of drops than would be the case if the distribution of the constituents were uniform. The relatively large volume of fluid required, and the more or less troublesome technic are further disadvantages in the application of the stalagmometer to biologic fluids. The method here described meets these objections, and is believed to possess advantages over other methods.

The procedure depends upon the rise of a fluid in a capillary tube. This principle was first made use of by Frankenheim,[1] in 1847, and then by Schiff,[2] in 1884, but unfortunately, as pointed out by Sugden, was overlooked until comparatively recent times. In 1915, Lord Rayleigh[3] published a discussion of the theory of the capillary tube. Sugden,[4] in 1921, and Richards, Speyers and Carver,[5] in 1924, described apparatus making use of the same principle, but instead of employing a single tube these authors employed two capillary tubes of different diameters, and measured the difference in level of the two menisci. This has been found to be more accurate. Moreover, the simplicity and ease with which the determinations can be made are striking. The method seemed applicable to biologic fluids. I have, therefore, made a simplified form of the apparatus described by Sugden, and by Richards, Speyers and Carver, and, in addition, simplified the technic of making measurements. The results obtained with this adaptation have been satisfactory.

THE CAPILLARY U-TUBE

Two capillary tubes of different diameters are sealed together and bent into a U-tube at the point of union so that the two arms are separated about one millimeter. The diameters of the tubes I have used are 0.7348 millimeters

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and 1.920 millimeters. A hole is blown in the bottom of the U-tube to permit entrance of the fluid. The apparatus is made small enough to slip readily into a Pyrex test tube one centimeter inside diameter, and seven to eight centimeters in length. Fig. 1 shows the essential details of the apparatus.

METHOD OF MEASURING SURFACE TENSION

From 0.5 to 1 c.c. of the fluid to be measured is placed in the dry Pyrex test tube. After thorough cleansing and drying, the capillary U-tube is immersed directly into the fluid, allowing it to rest in the test tube, and the

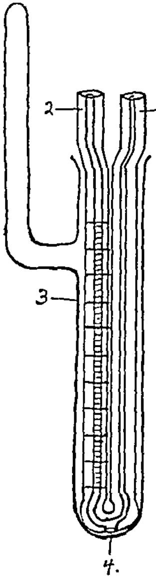

Fig. 1.—Capillary U-tube for measuring surface tension (drawn to scale).

1, Small capillary, radius about 0.4 mm.; *2*, large capillary, radius about 1.0 mm.; *3*, pyrex test tube with handle, inside diameter 1 cm., length 7 cm. to 8 cm.; *4*, opening in bottom of U-tube.

whole apparatus held vertically by a clamp on the handle in a constant temperature bath (a suitable beaker). When temperature equilibrium has been established, the fluid is drawn by suction through a small rubber tube attached to each capillary arm so as to wet the capillaries up to a height greater than the final level to which the liquid falls. The difference in level between the two menisci is read directly from the millimeter scale on the large tube, or measured by means of a pair of fine pointed calipers, which is then laid upon a millimeter scale. Readings are taken at two or three minute intervals until three successive readings give constant values. With pure

liquids, such as water, benzene, and alcohol, equilibrium, i.e., a constant level, is reached in about ten minutes. Serum and blood usually require more time.

RESULTS WITH WATER AND BENZENE

Using the apparatus described, measurements of surface tension were made on distilled water, and on a sample of Kahlbaum's benzene. The results are expressed as dynes/cm., being obtained by substitution in the following formula:

$$HGD = 2\gamma\left(\frac{1}{r_1} - \frac{1}{r_2}\right),$$

where H is the distance between menisci, G the gravitational constant, D the density of the liquid being measured, γ the number of dynes/ cm., r_1 the radius of the small capillary arm, and r_2 the radius of the large capillary arm. The radii were determined by the method employed by Richards, Speyers and Carver.[5] *The values obtained on distilled water and benzene are com*pared with values obtained by Sugden and by Richards, Speyers, and Carver in Table I. It is seen that the results obtained with the tube here described by me agree remarkably well with those reported by these authors.

TABLE I

SURFACE TENSION OF WATER AND BENZENE

	AUTHOR	SUGDEN	RICHARDS, SPEYERS, AND CARVER
Water	72.53 dynes/cm. at 20° C.	72.59 dynes/cm. at 20° C.	75.89 at 0° C.
	" " " " "	72.81 " " " "	70.72 " 34.32° C.
Benzene	28.49 dynes/cm. at 20° C.	28.86 dynes/cm. at 20° C.	31.87 at 0° C.
	" " " " "	28.83 " " " "	26.99 " 34.95° C.

BIOLOGIC FLUIDS

When surface tension measurements of serum are made by means of the stalagmometer it is to be expected that the observed value in dynes/cm. will be lower than the true value due to the collection in the surface of the drops of constituents lowering the surface tension. Comparison of values obtained by the stalagmometer with those determined by the capillary U-tube bear out this statement. For example, a sample of horse serum preserved with tricresol gave a value of 44.64 dynes/cm. with the capillary U-tube, and a value of 42.41 dynes/cm. with the stalagmometer. A repetition of the measurements on the same serum five months later gave 44.63 dynes/cm. with the capillary U-tube and 41.21 dynes/cm. with the stalagmometer. This discrepancy is not due to inability of the capillary method to check the stalagmometer because measurements of a sample of Kahlbaum's benzene gave 28.49 dynes/cm. with the capillary U-tube and 28.46 dynes/cm. with the stalagmometer.

Direct reading of the distance between menisci without employment of a cathetometer and reading telescope as used by Richards, Speyers, and Carver has given satisfactory results. Duplicate readings within 0.3 millimeter can easily be made. Using an apparatus having capillaries with the

radii of 0.3674 and 0.9600 millimeter and taking the values of G and D (water) as 980.4 and 0.9942, respectively, substitution in the above formula shows that a difference in level between the menisci of 0.5 millimeter corresponds to a surface tension change of 1.453 dynes/cm. Therefore, changes of at least 1.5 dynes/cm. in serum are certain to be detected with the apparatus and technic here described. The method has been successfully applied to blood, plasma, serum, and urine in this laboratory.

As illustrative of the application of the method to the detection of changes in surface tension produced by the addition of small quantities of substances to distilled water and to serum, the results in Table II are cited. The data are expressed as distance between menisci, a decrease in the distance corresponding to a lowering of surface tension and vice versa.

TABLE II

CHANGES IN SURFACE TENSION OF WATER AND BIOLOGIC FLUIDS PRODUCED BY BILE AND AGAR

Distilled water	25.0 mm.	Distilled water plus trace of bile salts	20.5 mm.
Horse serum	17.5 mm.	Horse serum plus trace of bile salts	15.0 mm.
Dog serum	17.0 mm.	Dog serum plus 0.5 mg. agar per c.c.	15.9 mm.
Oxalated pigeon blood	16.8 mm.	Oxalated pigeon blood plus trace of bile salts	16.0 mm.
Freshly voided urine	22.5 mm.	Freshly voided urine plus trace of bile salts	18.0 mm.

The advantages of the method are several and as follows: It is simple, easy, economical and accurate. Its automaticity eliminates the personal factor involved in other methods requiring drawing a wire loop through a drop or regulating the flow of a liquid. With biologic fluids errors resulting from evaporation and loss of carbon dioxide are reduced to a minimum.

SUMMARY

1. A simple apparatus and technic are described for the measurement of surface tension changes in pure and biologic fluids.

2. The method gives more reliable results with biologic fluids, such as blood serum, than are obtainable with the stalagmometer.

3. Measurement of the surface tension of distilled water and benzene gives results in good agreement with the known values for these substances.

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STUDIES IN LOCAL ANESTHESIA. IV*

The Pharmacology of Some Para-Amino-Benzoate Compounds

Local Anesthetic Action upon the Mucous Membranes and Skin of Man

By William R. Meeker, M.D., Chicago, Ill.

The merit of any substance proposed for local anesthesia is determined by the standards formulated by Braun, as follows: (1) the drug must produce a diffusible, complete, and lasting anesthesia; (2) following systemic absorption, it should be less toxic than cocaine in proportion to its anesthetic power; (3) it should not produce irritation and painful infiltration or cause local tissue damage, but should be absorbed without secondary effects, such as hyperemia, inflammation, exudation or necrosis; (4) it should be soluble in water and its solutions should be stable; (5) it should be readily sterilizable by heat, preferably by boiling in solution; (6) unless more powerfully anesthetic and at the same time less toxic than any known substances, the drug should be compatible in solution with adrenalin. For several years novocaine has most nearly fulfilled these requirements.

In the experimental determination of local anesthetic power, several methods have been proposed. The comparative value of these varies in the hands of different investigators and widely divergent results have been reported. In general, experiments performed upon laboratory animals show greater variation and are of less value than those performed upon man. Animal experimentation is of great value in the determination of toxicity and local effects upon tissues, but the ultimate efficiency of any new drug will then depend upon its action in practical use.

The purpose of the present investigation is to determine their anesthetic potential by experimentation upon the mucous membranes and skin of man.

A. SURFACE ANESTHESIA OF THE TONGUE BY IMMERSION

Anesthesia of the buccal mucous membranes was one of the earliest local anesthetic effects to be observed. The sensation of numbness resulting from chewing coca leaves suggested the use of cocaine as a surface anesthesia. The surface anesthetic power of new drugs may be demonstrated by applying them to the tongue or gums. The anesthetic action is manifested by a sensation of numbness very easily recognized. The experiment is essentially the same as clinical practice.

Method.—I have described the method elsewhere.† The tip of the tongue was immersed in solutions of the drug in physiologic saline for five minute peri-

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ods. When effective the tip of the tongue usually felt numb at the end of two minutes. The mouth was then rinsed out at the end of five minutes and the tip of the tongue tested for anesthesia by rubbing it against the cutting margins of the incisor teeth. Cocaine and butyn were used as controls. All tests were made upon my tongue. Sufficient time was allowed between tests for complete recovery of sensation when the solutions were anesthetic or when a distorted sensation resulted.

Results.—The comparative activity of these substances in both 2 and 1 per cent solutions is shown in the first two columns of Table I. The superiority of butyn to cocaine is at once manifest, the saturated solution of butyn (slightly less than 1 per cent) being as powerful as 2 per cent cocaine. Butyn is thus more powerful in surface anesthesia than any of the new drugs. A2 and B1 have no surface anesthetic power. B4 is anesthetic in saturated solution, which is between 0.25 and 0.5 per cent at room temperature. It is unfortunate that this drug is not soluble in higher concentrations as it is powerfully anesthetic. A5, B2, and B3 are anesthetic for a brief period in 2 per cent strength but ineffective in 1 per cent solution. A3, A4, A7, and B5 are easily the equals of

TABLE I

COMPARATIVE ACTIVITY OF DRUGS UPON THE TIP OF THE TONGUE. THE NUMBERS REPRESENT THE DURATION OF ANESTHESIA IN MINUTES

	THE DRUGS ALONE		2% BICARBONATE 2% OF DRUG
	2% STRENGTH	1% STRENGTH	
Cocaine	11	5-6.	
Butyn*		10.	
A2	0	0	
A3	11	6	11
A4	14	4	7
A5	5	?	10
A6	Irritating		
A7	12	4	8
B1	?	0	
B2	7	0	22
B3	5	0	18
B4†		5	
B5	11	5	22
B6	Irritating		

*Saturated solution somewhat less than 1 per cent.
†Saturated solution between 0.25 and 0.5 per cent.

cocaine. A6 and B6 were irritating in other experimental work. They exerted a biting, stinging effect upon the tongue lasting a few minutes, which further confirmed their irritant qualities.

The effect of the addition of sodium bicarbonate was next studied. In surface anesthesia it has been shown that the bases of local anesthetics are more effective than their salts. The addition of an equal amount of alkali to a novocaine solution increases the surface anesthetic power almost to that of cocaine. This is explained by the liberation of the free bases which penetrate the mucous membranes more readily than the water soluble salts.

Sodium bicarbonate was added to A3, A4, A5, A7, and B2, B3, and B5 in the same concentration as the anesthetic drug. With the exception of A5 and B2 all solutions became cloudy. A3, A4, and A7 became milky in appearance,

straw-colored, oily globules later floating on top. Focculi then appeared and later a curd-like precipitate. A4 and A7 were much less anesthetic at this stage and A3 just the same, thus showing incompatibility in solution with alkali.

The anesthetic power of B2 was markedly increased. B3 became opalescent but remained powerfully anesthetic. B5 was opalescent during the first six hours with a marked increase in anesthetic power. Twenty-four hours later oily globules separated out. While A4, A3, and A7 were incompatible with alkali after six hours, it is likely greater anesthetic power would have been observed just after the addition of the bicarbonate. The increase in anesthetic power by this means, when compatible, is roughly three times in terms of duration.

All tests for surface anesthesia of the skin were negative. The 2 per cent mixtures of the drugs with bicarbonate were applied to the skin for five minutes by means of a cotton applicator. The tests were repeated, dipping the applicator in crystals forming a paste. Finally the applicator was dipped in a mixture of equal parts of crystals of the drug and sodium bicarbonate. No anesthetic effect was produced by any of these methods.

B. ANESTHESIA OF HUMAN SKIN BY DERMAL WHEALS

Anesthesia of the skin by dermal wheals is an attractive method because it is identical with clinical usage. This method involves direct action on the terminal nerve filaments and sensory end-organs of the skin. Anesthesia is but very little dependent on pressure within the layers of the skin because wheals of physiologic salt solution do not produce anesthesia. Anesthesia, therefore, results from a direct chemical action upon the nerve endings. The great advantage of this method over others was first emphasized by Braun. He determined anesthetic potential both by duration and by minimal anesthetic concentration.

Method.—The thighs and anterior abdominal wall were closely shaved. Dermal wheals were then raised with the Meeker syringe and finest hypodermic needles freshly sharpened. The needle was thrust beneath the skin surface with the bevel downward. At the moment the needle point entered the epidermis, injection began, which was always endermic and not subcutaneous. Dermal wheals were thus made to stand up from the surrounding skin like urticarial wheals. Wheals were one-half of an inch in diameter and required one-fourth of a cubic centimeter of solution each. It is important that all wheals be as nearly the same size as possible and contain the same amount of solution, all of which has been injected intracutaneously. Adequate controls were employed, consisting of novocaine, cocaine, butyn, and salt solution, so that disturbance of sensation was not interpreted as anesthesia. Progressive series of dilutions in physiologic salt solution were injected, as one-fourth, one-eighth, one-sixteenth, one-thirty-second, one-sixty-fourth and one-one hundred and twenty-eighth of 1 per cent.

I made all wheals upon myself. The skin of the thighs and abdominal wall is of such thickness that accurate wheals may be raised painlessly when the substance is anesthetic. The sensitiveness of the skin and the rapidity of absorption vary in different areas of the body. It also varies in different individ-

uals, depending upon familial traits, exposure, vocation, etc. By employing the same skin areas in the same individual these factors remained constant. The duration of anesthesia in the same cutaneous area may also be shortened by previous brisk massage, heating, or muscular exercise because of the improved circulation and consequently more rapid absorption. In these tests the patient remained seated and sources of external heat were avoided. Wheals were marked with a circle of mercurochrome as soon as raised so that the center of the endermic infiltration was easily identified for testing after the wheal

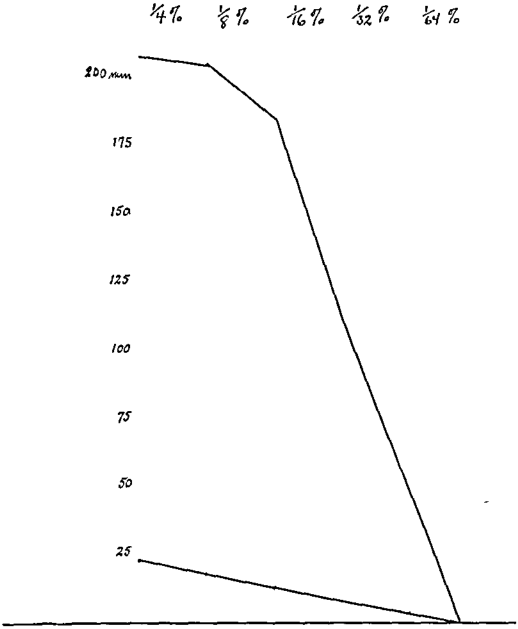


Fig. 1.—Diagrammatic representation of anesthetic power. Duration expressed by the base line of the isosceles triangle and minimal anesthetic concentration by the apex

had disappeared. Tests for sensation were made by scratching the area with a wooden applicator or with a needle as in vaccination.

Results.—Table II expresses both duration of anesthesia and minimal anesthetic concentration. The minimal anesthetic concentration of cocaine and butyn was one-thirty-second of 1 per cent. Both were ineffective in one-sixty-fourth per cent strength. The minimal anesthetic concentration of novocaine is one-sixteenth of 1 per cent. A2 is the equal of novocaine. A5, A6, and B1 are only slightly better. A3, B2, and B3 are the equals of cocaine. A4 is

slightly more powerful and A7, B4, B5, and B6 distinctly more powerful than cocaine and butyn. These relationships are expressed graphically in Fig. 1.

The stability of these drugs in solution was not thoroughly tested. They had been in solution a week before these tests were begun. After determination of anesthetic power in unsterilized solutions they were all boiled briskly for five minutes and the same tests repeated. No difference in anesthetic power was observed which was not easily within the limits of experimental error. The drugs will thus stand a limited amount of boiling in solution without deterioration.

TABLE II

COMPARATIVE ANESTHETIC ACTION IN DERMAL WHEALS. NUMBERS REPRESENT DURATION OF ANESTHESIA IN MINUTES

	1/4%	1/8%	1/16%	1/32%	1/64%	1/128%
Cocaine	21	16	10	6	0	
Butyn	22	15	11	7	0	
Novocaine	16	10	5	0		
A2	15	12	7	0		
A3	21	16	10	4	0	
A4	23	18	11	5	3?	0
A5	19	12	7	4	0	
A6*	16	10	7	4	0	
A7	23	20	18	11	5	0
B1	18	13	7	0		
B2	21	15	14	8	0	
B3	21	15	11	6	0	
B4	24	21	17	12	6	0
B5	25	21	14	10	4	0
B6*	25	21	15	10	4	0

*A6 and B6 are too irritating for clinical use.

TABLE III

COMPARATIVE ACTIVITY IN DERMAL WHEALS WITH THE ADDITION OF ADRENALIN, 10 MINIMS TO 100 C.C. OF SOLUTION*

	1/4%	1/8%	1/16%	1/32%	1/64%	1/128%
Cocaine	205	195	170	100	0	
Butyn	180	160	73	100	15?	0
Novocaine	155	130	50	0		
A3	160	140	95	50	0	
A4	180	170	150	90	60	0
A5	200	190	85	30	0	
A7	105	105	55	25	0	
B2	250	250	190	90	34	0
B3	200	210	200	80	20	0
B4	200	200	200	170	90	10?
B5	210	200	200	130	85	0

*The numbers represent duration of anesthesia in minutes.

Effect of Adrenalin.—Adrenalin by itself is not anesthetic, but added to solutions of novocaine and cocaine it increases the duration of the anesthesia from four to ten times. Tropacocaine is the only drug uninfluenced by the addition of adrenalin. Its action is not true synergism because the minimal anesthetic concentration is not altered. The effect has been repeatedly shown to be due to the vasoconstriction of the adrenalin, which has earned it the name of the chemical tourniquet.

Adrenalin solution (1:1000) was added to the anesthetic solutions in the proportion of 10 drops to one hundred cubic centimeters (three drops to the

ounce) just before using. When the control solutions had turned the slightest shade of pink they were discarded and fresh ones made.

The addition of adrenalin produced intense blanching in the wheals which often outlasted the effects of the anesthetic. The adrenalin in all instances greatly prolonged the anesthesia, as is seen in Table III. A2 and B1 were not included because of their lower anesthetic power. A6 and B6 were not included because of their irritating qualities. There is a greater variation in the end-point by this method so that it is not appropriate for quantitative comparisons of anesthetic power. Variations of from half an hour to an

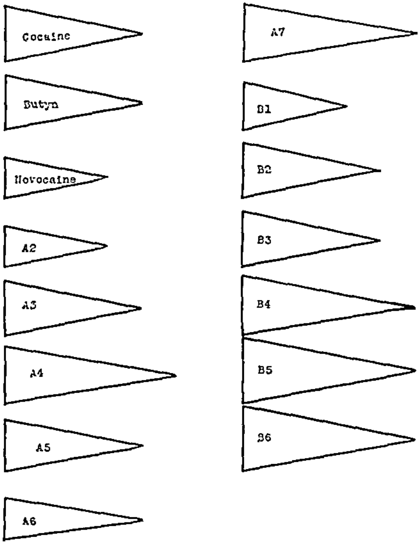


Fig. 2.—Curves representing anesthetic activity with and without adrenalin. Duration of anesthesia is shown on the vertical line and dilutions on the horizontal one. By the addition of adrenalin, anesthesia is prolonged 8 times and the more effective concentrations, ¼, ⅛, and 1/16 per cent tend to become equalized in duration.

hour are not uncommonly seen in repetitions due to unexplainable factors. These results would therefore show considerable variation if repeated.

The duration of anesthesia when adrenalin is used depends much less upon percentage strength of the drug, providing it is an effective concentration. The fourth, eighth, and sixteenth per cent strengths are very nearly equal in duration of anesthesia, for the same amount of adrenalin was used in each. Much weaker solutions should be employed in practical use when adrenalin is added. This relationship is expressed by the graphs in Fig. 2, which represents the average anesthetic action of A4, B2, B3, and B5, with and without adrenalin.

SUMMARY

The results of experiments thus far indicate that all these drugs are stable in solution, that they do not deteriorate by boiling for a limited time, and that they are compatible in solution with adrenalin.

A1 should be discarded because it has not sufficient anesthetic power. A2 and B1 may be discarded for the same reason, since they are no better in this regard than novocaine. (No. 2 is novocaine.)

A6 and B6 are unfit for practical local anesthesia because they are marked local tissue irritants.

For surface anesthesia of mucous membranes A3, A4, A7, and B5 are at least as powerful as cocaine, and give promise of usefulness in this field. B4 is not sufficiently soluble. The others have not sufficient penetrating power.

For infiltration anesthesia A3, A5, B2, and B3 are as powerful as cocaine. A4, A7, B4, and B5 are more powerful than cocaine, and promise to gain favor over the present drugs employed in this work. Their future usefulness in infiltration anesthesia will depend upon systemic toxicity and local tissue reaction. Further work along these lines is necessary before their fitness for clinical use is determined.

Local Tissue Reactions

By William R. Meeker, M.D., Chicago

Among the requirements which Braun[1] prescribes for a local anesthetic drug, a very important one is that it should not produce local damage to tissues. One of the common objections offered by the opponents of local anesthesia is the occasional interference with healing which they ascribe to the local action of the drug. Several proposed substitutes for novocaine have been more powerfully anesthetic but have failed to gain favor because of the local tissue irritation and damage which they produce.

The absence of irritating qualities in novocaine is marked even when applied in concentrated solution or powder form to sensitive wounds or on such delicate surfaces as the cornea. Injected within the tissues it exerts but little influence on the vasomotor nerves of the part. The injections are without pain and free from irritation. There is no after-pain, inflammation, persistent hyperemia, or induration.[2]

Cocaine may be ranked with novocaine in its action upon tissues. It produces vasoconstriction when applied locally and after injection into tissues, which results in blanching without preliminary burning or dilatation of vessels. Injections are painless and there are no harmful postanesthetic effects. When properly employed, therefore, the healing process is delayed only until the solutions are absorbed, after which it proceeds normally.

Quinine urea hydrochloride is an example of a local anesthetic which produces marked local tissue disturbances. Solutions of sufficient strength produce an anesthesia lasting for hours or even days, the prolonged anesthesia depending upon tissue damage. Hertzler[3] states that an exudate is produced

which is at first amorphous and later coagulates forming granular fibrin. A wheal thus produced becomes hyperemic and indurated, the fibrin being absorbed within one or two weeks with but slight replacement by fibrous tissue. Often in clinical work the parts assume a dark ecchymotic appearance which sometimes proceeds to coagulation necrosis and sloughing. Such a potential interference with healing is a serious drawback.

The local irritant action is also manifested by the production of pain during the injection. This is so marked that in rectal work Allen recommends the preliminary injection of Schleich's solution to prevent the burning pain during and immediately after the injection of quinine salts. An anesthetic which first elicits pain after injection has been called "anestheticum dolorosum." This is a manifestation of an irritant chemical reaction. For these reasons quinine urea hydrochloride has been largely abandoned for infiltration anesthesia and it never was popular in nerve block methods.

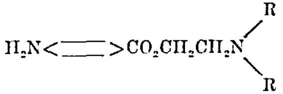
Alcohol is an example of a more marked tissue irritant or anestheticum dolorosum. Infiltration with it is exceedingly painful and the resulting anesthetic effect due more to destructive tissue change than to a specific anesthetic action upon nervous elements. Wheals of 50 per cent alcohol are followed by gradual sloughing of the center after a week or ten days.

NEW ANESTHETIC DRUGS

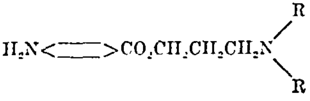
The drugs comprising the subject of this investigation were prepared and furnished by Dr. Roger Adams of the Department of Chemistry of the University of Illinois. They are divided into two groups, an ethyl and a propyl series, as follows:

	ETHYL SERIES	MOL. WT.
A-I	Dimethylamino ethyl para-amino benzoate hydrochloride	241.5
A-II	Diethylamino ethyl para-amino benzoate hydrochloride	272.5
A-III	Di-n-propylamino ethyl para-amino benzoate hydrochloride	300.5
A-IV	Di-n-butylamino ethyl para-amino benzoate hydrochloride	328.5
A-V	Di-isopropylamino ethyl para-amino benzoate hydrochloride	300.5
A-VI	Di-isobutylamino ethyl para-amino benzoate hydrochloride	328.5
A-VII	Di-secondary butylamino ethyl para-amino benzoate hydrochloride	328.5
	PROPYL SERIES	
B-I	Diethylamino propyl para-amino benzoate hydrochloride	286.6
B-II	Di-isopropylamino propyl para-amino benzoate hydrochloride	314.6
B-III	Di-normal propylamino propyl para-amino benzoate hydrochloride	314.6
B-IV	Di-normal butylamino propyl para-amino benzoate hydrochloride	342.7
B-V	Di-iso-secondary butylamino propyl para-amino benzoate hydrochloride	342.7
B-VI	Di-isobutylamino propyl para-amino benzoate hydrochloride	342.7

The general formula of the ethyl series is



That of the propyl series is



Other investigations have shown some of these preparations to be wanting in certain essentials which eliminates them as desirable anesthetics. A-I was found to be too weakly anesthetic for further consideration. A-VI and B-VI were found to be markedly irritating to rabbits' eyes even to the point of corrosion. Of the remainder A-II and B-I are least anesthetic, being no more powerful in this respect than novocaine. The present investigation, therefore, includes more particularly numbers A-III, A-IV, A-V, and A-VII and B-II, B-III, B-IV, and B-V.

The methods employed were the determination of: (1) Gross changes in dermal wheals on dogs, using the more concentrated solutions; (2) Gross changes following injections of the more concentrated solutions into rabbits' ears; (3) Gross changes and sensations produced by dermal wheals and irrigation of the conjunctival sac in man; (4) Microscopic changes following intradermal infiltration in the dog. Wherever possible controls of novocaine and cocaine were used because of their lack of irritant qualities, and quinine urea hydrochloride and alcohol were included because of their marked local irritation.

I. *Changes in the Skin of Dogs.*—Two series of dermal wheals were raised on the backs of two dogs. The solutions were of 2 per cent strength with the exception of B-IV which was a saturated solution. Within half an hour there was a yellowish discoloration of A-IV, A-VII, and the quinine urea hydrochloride. The center of the alcohol wheal was white surrounded by a hyperemic zone. One day later these wheals were marked by brick red discolorations resembling crusts, although they were really intracutaneous. Within ten days to two weeks these areas became crusts and were later cast off representing a superficial necrosis. Hyperemic areas persisted after the desquamation of these crusts. These changes were more marked with alcohol, decreasing in severity with A-VII, A-IV, and quinine urea hydrochloride, in the order named. There was no manifestation of local injury with the other drugs in this test.

II. *Injections into rabbits' ears* with 2 per cent solutions proved valuable in determining the amount of induration as well as other gross changes. One-half of a cubic centimeter of 2 per cent strength was injected in each case. After an interval of a day A-IV and A-VII were characterized by marked thickening. The same feature was observed to a less extent with quinine urea hydrochloride, butyn, and B-V. These areas were gradually resorbed within ten days without necrosis.

III. *Dermal Wheals and Conjunctival Irrigations in Man.*—The use of dermal wheals on man is a convenient method of studying local irritation as well as anesthetic power, providing it is already known from animal experimentation that the concentrations employed will not be escharotic. I placed two series of wheals of 2 per cent strengths upon my thigh. A-IV and A-VII became discolored as in the dog. In addition the stratum corneum became raised after twenty-four hours to form fair sized blisters. Later the areas became brick red in color forming crusts after two weeks which persisted beyond the limits of these observations. This superficial necrosis was more marked with 50 per cent alcohol, crusts of which were case off after three weeks. Such changes are less marked with quinine urea hydrochloride.

Painful infiltration was marked only with alcohol, quinine urea hydrochloride and to a much smaller degree with A-IV. In order to accentuate the pain-producing qualities, solutions were made in distilled water. Infiltration with these solutions was painless even in hypotonic solution, as one-fourth per cent strength. All of the new drugs are therefore true anesthetics and cannot be classed with quinine and alcohol as anesthetica dolorosa.[4]

One-half per cent strengths were used in irrigations of the conjunctival sac. A-IV and A-VII were markedly irritant. Irrigation was followed at once by a burning, smarting, and aching pain until the solutions were washed out. Marked injection of the conjunctiva persisted. There was mild transient smarting with quinine urea hydrochloride, which was less marked with butyn and B-V. Very slight hyperemia followed B-V. The others were entirely negative.

IV. *Microscopic Changes.*—Dermal wheals of large size were raised with 2 per cent strengths in salt solution usually injecting one-half of a cubic centimeter to each wheal. After twenty-four hours the dog was killed and the wheals excised. Microscopic sections were made according to the usual pathologic technic and stained with hematoxylin and eosin.

All wheals were somewhat indurated to palpation, A-IV and A-VII being more marked. In man the induration of these two wheals and those of quinine and butyn lasted longer than that of the other drugs.

The epithelial layers of the epidermis were apparently normal. While in man it is necessary to infiltrate between the epithelial layers to produce the charactristic raised, orange peel appearance of a wheal, the epidermis is much thinner in the dog so that infiltration between the epidermis and corium produces typical raised wheals. The infiltrated area proper included only corium, extending from the epidermis as deep as the bases of the hair follicles. The infiltrated area was surrounded by a zone of reaction comprising contiguous subcutaneous tissue. Three separate layers or zones were thus recognizable in all wheals. From the surface these layers were: (1) epidermis, (2) infiltrated area of the corium, and (3) the reaction zone of surrounding subcutaneous tissue.

The infiltrated area was easily recognized in all wheals. Tissue spaces seemed larger than normal, fibers being spread apart and arranged in loose meshwork. Individual strands were much enlarged, probably from imbibition of infiltrated fluid, and the entire area stained a dark pink color. There was a general hyaline appearance in which the finer structural details were lost.

Tissue changes varied from complete superficial necrosis to only a slight round cell infiltration. Cocaine and novocaine showed no coagulated exudates in the infiltrated zone and only a mild lymphocytic infiltration in the subcutaneous zone of reaction. In both instances a few lymphocytes had found their way into the infiltrated area. These are examples of mild tissue reactions.

Butyn, quinine urea, and alcohol produced more severe local tissue changes. In the butyn wheal the connective tissue strands were more thickened. There was a very marked infiltration of lymphocytes in the surrounding subcutaneous tissue, which also permeated the area of anesthetic drug infiltration. These changes were equally as marked in the quinine urea wheal and there were also small granular areas of coagulated exudate in the reaction zone of the subcu-

taneous tissue. There was marked hyalinization of the infiltrated zone of the wheal of 50 per cent alcohol. A fine granular material representing coagulated exudate was also present between strands of fibrous tissue. Cellular infiltration was not marked.

Local tissue disturbance may thus be estimated upon (1) the swelling and hyalinization of connective tissue strands of the infiltrated corium; (2) the presence of transudates and exudates; (3) the infiltration with lymphocytes and leucocytes. The extent of irritation of the new drugs may be estimated as follows:

A3 +++.	B2 +++.
A4 +.	B3 +.
A5 ++.	B4 ++.
A7 +++.	B5 +++.

SUMMARY

The criteria of tissue disturbances employed show considerable variation in irritant action. All the new drugs tested were true local anesthetics in that anesthesia was never preceded by pain during infiltration. Gross tissue changes of dermal wheals show A-IV and A-VII to be sufficiently corrosive to produce superficial necrosis. This action was also manifest on rabbits' ears; B-V also appeared irritating. In the conjunctival sac one-half per cent solutions of A-IV and A-VII were markedly irritating and B-V mildly irritating. The other drugs caused no disturbances. Microscopic study of excised wheals further confirms the irritant action of A-VII and B-V and show A-III and B-II to be equally disturbing.

A consideration of all tests warrants the discarding of A-IV, A-VII, and B-V. It seems certain that these drugs would cause interference with healing in clinical practice. Which of the remaining drugs deserve a clinical trial will depend upon systemic toxicity. This is of extreme importance in many of the regional anesthetic procedures where the injection of large amounts of the anesthetic drug is necessary. The low toxicity of novocaine has been largely responsible for the development of regional anesthesia to its present high status. Other features being equal, the drug replacing novocaine in regional anesthesia must either be more powerfully anesthetic in proportion to toxicity, or less toxic in proportion to anesthetic power, thus affording a wider margin of safety when large amounts must be injected.

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STUDIES IN LOCAL ANESTHESIA. V

The Toxicity of Para-Amino-Benzoate Compounds*

By H. McGuigan, Ph.D., M.D., and Glenn A. Brough, M.S., M.D., Chicago, Ill.

THE following experiments were carried out to determine the toxicity of the compounds of this series by injection into the peritoneal cavity of albino rats. This was chosen because rats were available, and also because the conditions approximate clinical application more nearly than if cold-blooded animals had been used. The method used was as follows:

The rats were weighed. The dose was given in milligrams per kilogram body weight. Since the solutions were equimolar, the same volume of each solution in cubic centimeters was given to rats of the same weight. The molecular concentration corresponded to 1 per cent procaine. The solutions were kept at body temperature by an electric thermostat, and all intervals were timed with the stop watch.

The animal was held; injection was made into the peritoneal cavity, and the animal immediately turned loose. The symptoms of toxicity were practically the same in all cases, regardless of the drug used. The rat soon became restless, excitable, and decidedly irritable. A touch on the tail at times caused a very definite jump. There were four stages in the symptoms as follows: first, increased irritability; second, paralysis; third, convulsions; fourth, coma and death. The onset of each of these was more or less abrupt, and in the case of the convulsions and death was easily determined.

The rats after the short irritable excitable stage, began to show signs of paralysis. The wheezy dyspneic character of the respiration, accompanied by gnashing of the teeth, became more and more marked, while the hind legs became slowly paralyzed. Although the legs were motionless, a painful stimulus caused the rat to squeal and attempt to drag them away. The other reflexes throughout were positive. The clicking or gnashing of the teeth and the dyspnea continued, the neck became arched backward, and the animal suddenly went into a convulsion which lasted a few seconds, and was followed by another after a short interval. During the spasm the animal was in opisthotonos, but in the interval it relaxed with the exception of the neck muscles. This point of relaxation was not true in the later stages, where exhaustion seemed to supervene. In some instances when large fatal doses were given, the spasms were tonic instead of clonic in type. They usually ended in death without remission. The accessory respiratory muscles were active after the breathing became difficult.

Death occurred from a respiratory failure, the heart continuing to beat for about one minute after cessation of respiration. Thus two definite points

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were taken as a measure of the relative toxicities—the time of the onset of the convulsions, and the time of death.

Animals which survived the convulsions for periods of twenty to thirty minutes usually recovered, regardless of how severe the convulsions had been, or the length of the coma folowing the convulsions, which might have been an hour or more. The following morning the rats were apparently normal. In some cases weakness of the hind legs was marked and persisted for twenty-four hours or longer.

Table I gives (A) the dose that will cause convulsions, (B) the fatal dose, (C) the numbers of the drug which cause these symptoms with any given dose.

TABLE I

DRUG A	MINIMUM MG. PER KILO CAUSING CONVULSIONS	AV. TIME IN MIN. OF ONSET OF CONVULSIONS.	NUMBER OF ANIMALS USED	NUMBER HAVING CONVULSIONS	MINIMUM MG. PER KILO CAUSING DEATH	AVERAGE TIME IN MINUTES—SECONDS	NUMBER USED	NUMBER OF DEATHS
I	350	7'45"	3	1	non toxic		3	0
II	150	5'25"	17	7	150	6'45"	17	5
III	75	3'50"	4	3	100	4'15"	10	5
IV	25	8'00"	3	1	50	7'20"	5	2
V	75	4'05"	2	1	100	3'10"	5	1
VI	75	2'50"	2	1	100	5'10"	4	2
VII	75	2'25"	2	1	100	3'00"	4	2

TABLE II

SERIES B. (BY S. J. COHEN)

	DOSE PER KILO THAT KILLS BY CARDIAC AND RESPIRATORY PARALYSIS	TIME TO KILL		DOSE PER KILO FROM WHICH THEY RECOVER
		HR.	MIN.	
B 1	125 mg.	5	00	100 mg.
B 2	75 mg.	4	15	50 mg.
B 3	100 mg.	7	25	75 mg.
B 4	75 mg.	2	55	50 mg.
B 5	100 mg.	2	50	75 mg.
B 6	200 mg.	15	00	150 mg.
order				
B 4	75 mg.	2	55	
B 2	75 mg.	4	15	
B 5	100 mg.	2	50	
B 3	100 mg.	7	25	
B 1	125 mg.	5	00	
B 6	200 mg.	15	00	

TABLE III

MINIMAL LETHAL DOSE OF COCAINE IN WHITE RATS. INTRAPERITONEALLY (COHEN AND McGUIGAN)

NUMBER OF RATS USED	AMOUNT OF DRUG	TIME OF CARDIAC STOPPAGE		REMARKS
		HR.	MIN.	
2	100 mg. per k.	4	50	Both died
1	75 mg.	3	05	Died
6	50 mg.	6	06	3 died 3 recovered
2	25 mg.	Recovered		Both recovered

TABLE IV

	SERIES A					COCAINE EQUIVALENTS (COCAINE = I)		
	DURATION OF ANESTHESIA IN RABBIT'S EYE	QUADDEL METHOD. AMT. OF DRUG IN 1 C.C. TO PRODUCE ANESTHESIA IN 5 MIN.	TIME NECESSARY TO PRODUCE PARALYSIS OF THE SCIATIC NERVE IN FROG	PHENOLIC COEFFICIENT. TIME NECESSARY TO KILL BACTERIA IN 1% SOLUTION	TOXICITY. MINIMUM AMT. PER KILO BODY WEIGHT OF DRUG PRODUCING DEATH IN WHITE RATS	COCAINE VALUES IN EYE	ON SCIATIC NERVE PARALYSIS	ON FROG'S SKIN (TÜRCK METHOD)
I	none	–	13′ 57″	–	nontoxic	none	0.25	0
II	14 min.	10 mg.	5′ 30″	9 hr.	150 mg.	0.20	0.16	0
III	16 min.	7 mg.	1′ 42″	5 hr.	100 mg.	0.25	1.7	1
IV	30 min.	19.5 mg.	1′ 30″	2.5 hr.	50 mg.	0.62	2.0	0.8
V	23 min.	15 mg.	1′ 5″	4.5 hr.	100 mg.	0.44	3.0	0.4
VI	45 min. very irritant	12.5 mg.	3′ 55″	(0.15%) 5 min.	100 mg.	2. +	0.25	0.3
VII	46 min.	7.0 mg.	2′ 0″	45 min.	100 mg.	2. +	2.0	0.6
Cocaine		6.0 mg.			50 mg.			

TABLE V

	SERIES B					COCAINE EQUIVALENTS		
	DURATION OF ANESTHESIA IN RABBIT'S EYE	QUADDEL METHOD. AMT. OF DRUG IN 1 C.C. TO PRODUCE ANESTHESIA IN 5 MIN.	TIME NECESSARY TO PRODUCE PARALYSIS OF THE SCIATIC NERVE IN FROG	PHENOLIC COEFFICIENT. TIME NECESSARY TO KILL BACTERIA IN 1% SOLUTION	TOXICITY. MINIMUM AMT. PER KILO BODY WEIGHT OF DRUG PRODUCING DEATH IN WHITE RATS	COCAINE VALUES IN EYE	ON SCIATIC NERVE PARALYSIS	ON FROG'S SKIN (TÜRCK METHOD)
I	26 min.	20 mg.	2′ 32″	2.5 hr.	125 mg.	0.50	0.5	0
III	35 min.	18.5 mg.	2′ 8″	3 hr.	100 mg.	4.	0.4	0.7
IV	42 min.	2.5 mg.	1′ 15″	(1/3%) 5 min.	75 mg.	0.75	0.9	0.2
II	60 min.	15 mg.	3′ 0″	3 hr.	75 mg.	1%	2.7	1.0
V	63 min.	17 mg.	1′	(0.5%) 5 min.	100 mg.	4	1.3	0.9
VI	Too irritant	21 mg.	2′ 20″	(0.2%) 5 min.	200 mg.	Too irritant for use	0.7	0.9

There is some slight variation in the tables which is very obviously due to differences in the rate of absorption, e.g., the doses causing convulsions under (C). No. 4 is in the first place in each case (except with 75 mg. per kilo) when Nos. 7 and 3 are shown to cause convulsions before No. 4. In such cases as this, it is due we think to slight differences in the rate of absorption.

RÉSUMÉ AND CONCLUSIONS

The results of the various methods are in general agreement that the anesthetic power of an homologous series increases with the molecular weight. The results recorded in this investigation do not always agree with this statement, but there is little reason to doubt the validity of it. The variations are to be explained mainly on variations in the rate of absorption. In such cases if a sufficiently large number of experiments were carried out the average would follow the general rule. Where the conditions can be more rigidly controlled as in the conjunctival sac, and in the determination of the phenolic coefficient, increasing action with increasing molecular weight is obvious. The toxicity as determined by injecting a solution of the drug into the peritoneal cavity is less than cocaine and somewhat greater than novocaine. The increase in toxicity, however, is less than the anesthetic action. As a result of this study Tables IV and V, the following numbers would seem worthy of clinical investigation.

A or ethyl series—III, IV, V, and VII.

B or propyl series—II, IV, and V.

Phenolic Coefficient*

By M. B. Burns, B.S., and N. Bullock, B.S., Chicago

The phenolic coefficient was obtained by putting typhoid bacilli in a strength of the solution, and determining how soon death took place in this solution.

Table I

Phenolic Coefficient (Arranged in Order of Efficiency)

DRUG	PERCENTAGE OF SOLUTION NECESSARY TO KILL	TIME NECESSARY TO KILL TYPHOID
A 2	1	9 hr.
A 3	1	5 "
A 5	1	4½ "
B 2	1	3 "
B 1	1	2½ "
B 3	1	1½ "
A 7	1	45 min.
Phenol	1	5 "
A 4	1	2½ "
B 5	0.5	5 "
B 4	0.33	5 "
B 6	0.20	5 "
A 6	0.15	5 "

The order of toxicity of the ethyl and propyl series is as follows:

ETHYL SERIES	PROPYL SERIES
2	2
3	1
5	3
7	5
4	4
6	6

A great number of determinations were made, and Table I gives the final results. The determinations were made as follows:

Five c.c. of the anesthetic solution were inoculated with one loopful of a twenty-four-hour growth of typhoid, and this was repeated with various dilutions until the minimal strength which would kill typhoid growth was determined. When no growth on broth was obtained by reinoculation from this solution, it was considered that the limit had been reached. A large series was determined, and in Table I the minimal lethal concentration with the time necessary for the germs to sojourn in this solution was recorded. The aim was to determine the time of death in 1 per cent strength, but some of the solutions were so active that dilutions weaker than this had to be made in order to get the relative toxicity. Table I is self-explanatory and gives the order of strength of the toxicity of these drugs.

A FILTERING CYLINDER AND CULTURE TUBE FOR BACTERIOLOGIC WORK*

By Eleeza Ablahadian, B.S., Glendale, Cal.

THE customary method of *cotton plugging* flasks, test tubes, burettes, etc., is by wrapping the cotton in a piece of gauze and plugging the flask which is to be sterilized and saved for later use. When flasks containing solutions are subsequently opened, some cotton fibers often float around and fall into the solution. My first thought was to turn a beaker upside down over the mouth of the flask, without cotton. This method is satisfactory if the beaker fits well, and the flask is not to be shaken much. (Fig. 2.)

It is, of course, a development of the Petri dish idea; but, it is not always easy to find beakers that will fit the flasks.

I therefore moved the cotton from the mouth of the flask to the neck. (Fig. 3.) If the cotton is wrapped tightly around the neck of the flask, and if the beaker is clean and dry to begin with, during sterilization the cotton will stick to the flask, but not to the beaker, which is fitted over the cotton rather loosely. In opening the flask, remove the beaker carefully, holding it mouth down, pour the solution out, pass the beaker over a flame, flame the mouth of the flask without touching the cotton, and replace the beaker. There will be no scorched bits of cotton floating around, sticking to the mouth of the flask, or falling into the solution.

Using the Petri dish idea again, I devised a *culture test tube.* (Fig. 4.) This test tube has a groove about three-fourths of an inch from the mouth. Cotton is wrapped around the groove and a glass cap is inverted over the mouth of the test tube, covering the cotton. The cap has a flat knob, so that when necessary to open the test tube, it may be held between the left thumb and forefinger, while the test tube is held between the fore and middle fingers.

*From the Research Laboratory, Harrower Foundation Clinic, Glendale, Calif.
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The right hand manipulates the platinum rod. The cap may be sterilized before and after operation, and replaced. Cultures keep well in these tubes.

THE FILTERING CYLINDER

Those who do a great deal of vacuum filtering, using small quantities at a time, know what a troublesome process it is. Usually, a sterile test tube is placed inside the filtering flask to collect the filtrate. It is troublesome to

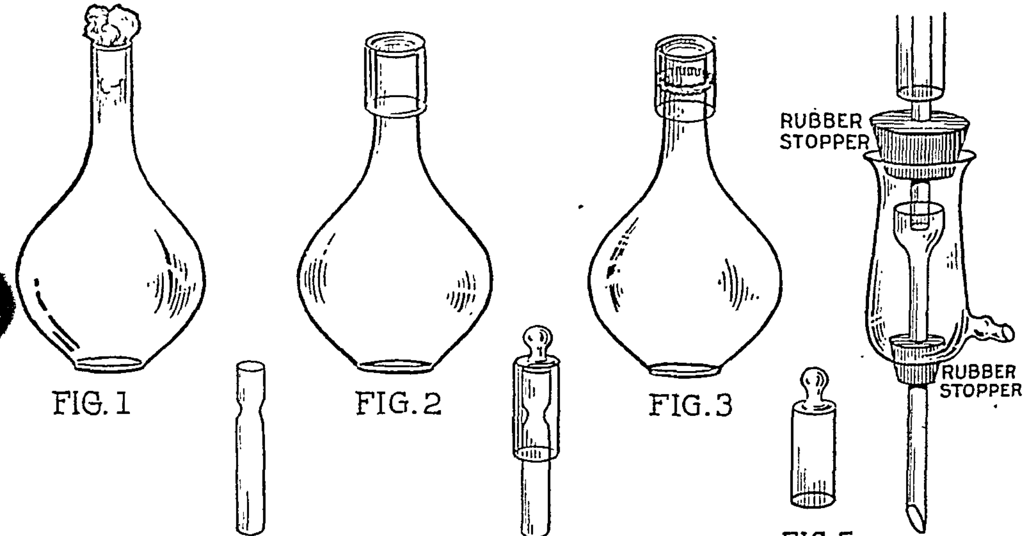

FIG. 1 FIG. 2 FIG. 3 FIG. 4 FIG. 4 + 5 FIG. 5 FIG. 6

stop the operation, take everything apart, remove the test tube when full, empty it, replace, and start filtering again. At the same time, there is the risk of contaminating the filtrate.

With a filtering cylinder, once the filter is placed and vacuum started, the process can go on without interruption. If a water pump is used, the cylinder takes care of the back-flow, and there is no danger of water getting into the filtrate. The receiving flask or bottle, placed at point "A," can be of any size, and may be removed without touching the rest of the apparatus.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

Coca, A. F., and Milford, E. L.: The Preparation of Fluid Extracts and Solutions for Use in the Diagnosis and Treatment of Atopic Conditions. Jour. Immunol., March, 1925, x, ii, 555.

Coca and Milford report a continuation of their studies in this subject, this being the seventeenth paper of a series.

The paper, though brief, contains a wealth of information and should be in the files of every worker engaged or interested in this work.

They find that, except for pollen extracts, dialysis offers a ready means of purifying many of the extracts with resultant increase in activity and stability.

Dialysis is always carried out against the same solution as was used for extraction, using the largest caps supplied by the Capes-Viscose Inc. Co. (132 Madison Ave., New York). Perfect antisepsis can be secured by a little toluol inside the cap. The process is carried out at room temperature, the outside fluid being changed once or twice daily for, usually, six days.

For all materials except pollens the extracting fluid is prepared as follows:

Two stock solutions are made up:

I.	KH_2PO_4	3.63 grams
	Na_2HPO_4	14.31 grams
	NaCl	50 grams
	Distilled water to make 1000 c.c.	
II.	Carbolic acid 4 per cent.	

To equal parts of these fluids add four parts of distilled water. The resultant mixture is the buffered saline extracting fluid (B.S.), and has a P_H of 7.0. It can be boiled or autoclaved without appreciable change in reaction.

The phosphate salts can be obtained from the J. T. Baker Co. and it is important to specify that they are to be used in the preparation of buffered solutions.

For pollen extractions Coca's original fluid is still used.

The formula for this (Jour. Immunol., March, 1922, vii, No. 2, p. 163) is:

Sodium chloride	0.5 per cent.
Sodium bicarbonate in such concentration that 10 c.c. of the fluid equaled about	3 c.c. of n/10 alkali.
Carbolic acid in final concentration of	0.4 per cent.

Solution should be made without the use of heat or excessive shaking.

For the cleansing of Berkefeld filters so that a neutral reaction results the following procedure was found satisfactory:

Mechanical cleansing with a "not too stiff bristled handbrush" is followed by immediate boiling for fifteen minutes in 2 per cent washing soda. Rinse in tap water and again boil. *Ten to twenty filters are then boiled in 2 liters of distilled water to which has been* added 50 c.c. of a 1:100 dilution of concentrated HCl. After two further boilings in plain distilled water, the filters are set up as filtering flasks and 300 to 400 c.c. of distilled water drawn through each. They are then wrapped in muslin and autoclaved.

Detailed and minute directions are given for many preparations. They do not lend themselves well to condensation or abstraction and the original paper should be consulted for the method of treatment of pollens, horse dander, feathers, rabbit, guinea pig, goat, cat, and dog hair, cow dander, nuts, seeds, and spices.

A new observation is recorded concerning milk as a result of which the authors no longer separate the proteins as test materials. It was found that raw, fresh milk from which the cream had been thoroughly separated by a Sharpless centrifuge could be filtered through a Berkefeld (N) filter. Such filtered milk remains stable for two to three months and is used for testing in 1:10 to 1:100 dilutions.

The methods described in the paper have given extracts remaining stable for at least a year in the majority of instances.

Following the observation that a quantity of suspended material, assumed to be largely desquamated epithelial cells, was present in the washings of feathers, and the substantiation of the assumption that the active substances were contained in these scales, the authors now routinely use an extract of this material in testing feather sensitive individuals.

In the preparation of hair extracts only the suspended particles in the ether washings are used, the hair being discarded.

The paper should be consulted in its original form for a full appreciation of the data contained.

Seyderhelm, R.: The Testing of the Vitality of Isolated Cells by Means of Colloidal Dyes. Deutsch. med. Wchnschr., Jan. 30, 1925, li, 180.

Cellular death is marked by the nuclear absorption of a colloidal dye; injured cells ermit the slow passage of the dye through their injured membrane.

A mixture of congo red and trypan blue (strength not stated) has been applied by the uthor to the study of urinary sediments.

After centrifugation a drop of the sediment and a drop of the mixed dye solutions re examined under a cover-glass.

Leucocytes from inflammatory foci remain unstained; pus cells (dead leucocytes) are stained red or with a tinge of violet. Vaginal and bladder epithelium are usually mottled. Casts show marked variations, sometimes dependent upon the vitality of adherent cells.

Waxy casts are deep blue; hyalin, variable. Mucus is red.

It is suggested that this "staining of degeneration" could be extended to other materials as exudates, cerebrospinal fluid, etc.

Cornwall, L. H., and Peck, S. M.: Three Cases of Granuloma Inguinale with an Investigation Concerning the Etiology. Proc. New York Path. Soc., xxxv, 1-5, p. 43.

The authors report that the organism demonstrated in their cases presents a very characteristic appearance on Sabauraud's medium—4 per cent maltose peptone agar. The size is variable—1 to 6 micra long by 0.5 micron wide, one end rounded and the other tapering to a point. With the Gram stain there is a definite cell membrane and some pleochromism. The cytoplasm contains from 1 to 5 purplish granular bodies. Occasional bipolar bodies or beaded forms are seen and oval, circular, or polyhedral forms are encountered. These characteristics are lost after 180 hours on this medium and are restored with great difficulty, if at all, by subcultures which revert to the diplobacilli, diplococci, bacillary, and coccoid forms usually seen on plain agar.

The authors consider the organism entirely distinct from B. mucosus capsulatus.

Morris, W. H.: The Value of Erythrocyte Sedimentation Determinations in Pulmonary Tuberculosis. Am. Rev. Tuber., December, 1924, x, 431.

The technic adopted [illegible] author is as follows:

Blood is collected [illegible] ge containing sufficient 3.8 per cent sodium citrate to give a 1:4 dilution of the b[illegible] blood is placed in small Wassermann tubes.

Calibrated 1 c.c. p[illegible] filled to the 1 c.c. mark, stoppered with a bored-out rub[illegible]ork, and placed in [illegible] om temper[illegible] Readings are made at one, two, and [illegible]-four hours, repre[illegible] ages of [illegible] volume of the blood. [illegible]ormal findings in [illegible] e 1 to [illegible]; in the female, 5 to 10 per cent.

Studies were made on 150 patients in varying stages of tuberculosis. The conclusions were:

1. Sedimentation velocity is increased in active pulmonary tuberculosis.
2. There is no constant relation between the extent of the lesion and the velocity of sedimentation.
3. The test offers a valuable estimate of the degree of activity.
4. The test has little value in diagnosis.

Zecker, T. T. and Goodell, H.: The Sedimentation Rate of Erythrocytes. Am. Jour. Med. Sc., February, 1925, clix, 209.

Two centimeters of 3 per cent sodium citrate are placed in a 15 c.c. graduated centrifuge tube and blood added to the 10 c.c. mark.

The tube is inverted and allowed to stand and the height of the red cells read at one hour. If the plasma is slightly higher or lower than the mark, the volume of red cells is corrected accordingly.

Studies were made of 125 cases. Readings were as follows:

Tuberculosis and acute inflammatory conditions, 3 to 6 c.c.; malignant tumors, 2 to 7 c.c.; pregnancy, 3 to 8 c.c.; all other conditions, 4.5 to 9.8 c.c.

Average normal readings were 7.1 c.c.

The authors recommend serial determinations and emphasize the necessity for correlation of the test with the other clinical and laboratory findings.

Julianelle, L. A., and Pons, C. A.: A Study of the Serologic Reactions Associated with Experimental Plethora and Plethoric Anemia. Jour. Clin. Investig, August, 1925, i, vi, 519.

Experimental studies have shown that following the artificial production of plethora an anemia occurs the mechanism of which has not been explained.

Explanations considered probable embrace: (a) the development of isohemolysins, and (b) destruction of transfused blood by increased activity of hemophages or through an increase in the number of such cells.

The authors report studies devoted to the demonstration of these possibilities.

Artificial plethora was induced in rabbits by the daily injection of rabbit blood (mostly citrated).

Incompatibility was never encountered. Plethora occurred, as a rule, in four to seven days and varied from 30 to 100 per cent increase in cells and from 20 to 50 per cent increase in hemoglobin. A gradual fall then occurred in spite of repeated transfusions, the fall stopping in some cases when reduction reached the normal level, in others progressing to a true anemia.

The red cells dropped before the hemoglobin, the graph being generally a straight line. The hemoglobin graph was step-like.

The reticulocytes varied inversely as the red cell count. The leucocytes fluctuated irregularly. Plethora was associated with lymphocytosis; anemia with a decrease in lymphocytes in favor of the neutrophiles. Rarer forms of white cells were infrequent.

The changes were the same regardless of whether or not the blood was citrated.

Marked congestion of the spleen and liver; intra- and extracellular hemosiderin deposits in large amount; endothelial hyperplasia; absence of changes in the lymph follicles; congestion and casts in the kidneys; and engorgement of capillaries with cellular debris and hemosiderin were the salient autopsy and microscopic findings.

Isohemolysins were not demonstrated either in vitro or in vivo.

Isohemagglutinins were found in half the animals but bore no relation to the degree of blood destruction.

Evidence of phagocytic or hemophagic activity was not demonstrated in fixed tissues. There was no evidence of any potential grouping in the rabbit blood.

The explanation of postplethoric anemia awaits further investigations.

Pilot, I., and Meyer, K. A.: Fusiform Bacilli and Spirochetes XII: Occurrence in Gangrenous Lesions of the Finger. Arch. Dermat. and Syph., December, 1925, vi, 837.

The previously reported studies of Pilot and Pilot and Davis have demonstrated the organisms first associated with Vincent's angina as very widespread invaders responsible for a variety of pathologic conditions.

In the present report Pilot and Meyer report their occurrence as causative agents in gangrene of the finger in a patient addicted to nail-biting, the organisms undoubtedly having been transferred by this habit.

The condition was treated successfully by neoarsphenamine.

The article is illustrated.

Schobl, O., and Ramirez, J.: The Fallacy of the Test for Lactose Fermenters as an Indicator of Fecal Pollution of Waters. Philippine Jour. Med. Sc., July, 1925, xxvii, 317.

The authors endeavored to determine, in view of the importance attached to lactose fermenters as an indication of fecal pollution in water, whether or not these forms are indisputably indicative of fecal contamination and, also, whether the representatives of the B. coli group are limited to human and animal excreta predominantly or if B. coli of human origin can be differentiated from B. coli of animal origin.

Studies were made upon well waters in Manila in the course of which it was found that parts of the pump which were constructed of organic material, such as hemp and leather washers, harbored large numbers of lactose fermenters. The same findings were noted in the bacteriologic examination of fresh, unused parts.

Other examinations disclosed lactose fermenters as nearly ubiquitous, being present in dust, grass, leaves, and shrubs.

Their presence in water, therefore, cannot be taken as final evidence of fecal pollution and should not serve as the sole basis for the condemnation of a water supply.

Specimens of feces were examined from various sources: fish, frogs, shrimp, water insects, horse, goat, carabao, rabbit, guinea pig, white mice and rats, rooster, owl, and man—in the endeavor, which was unsuccessful, to find some points serving to differentiate colon bacilli of animal and human origin.

The conclusions formed from the work follow:

1. Lactose fermenters as a group cannot be considered as an indication of fecal pollution.

2. True B. coli can be differentiated by the type of colonies on eosin-methylene-blue-lactose-agar plates.

3. There are no criteria for the differentiation of B. coli of human origin from those of animal origin.

4. The pollution of water by B. coli from the feces of fish or water-living animals is unlikely under ordinary circumstances.

Fischel, K.: The Suspension Stability Rate of Erythrocytes in Pulmonary Tuberculosis and Its Significance in Artificial Pneumothorax. Am. Rev. Tuber., January, 1925, x, 606.

Commenting upon the variations in technic associated with this procedure, the author proposes a method of calculating the reading which eliminates the necessity for specially calibrated tubes, etc.

It is based upon the principle of constant height rather than constant quantity of blood as suggested by Westergren.

Tubes of $\frac{2}{8}$ to $\frac{3}{16}$ inch diameter are used and the results expressed by calculating the height of the clear column in percentage of the whole suspension column.

A 2 c.c. syringe containing 0.4 c.c. of 3.8 per cent sodium citrate is filled with blood to the 2 c.c. mark. The syringe is inverted and the blood placed in the tube, which must be clean and dry. The tube is placed in a rack at room temperature and readings are made at one, two, six, and twenty-four hours.

The height of the whole column in millimeters is divided into the height of the plasma column, the quotient giving the percentage reading.

The paper contains numerous tables and graphs and, on the whole, confirms the investigations of previous workers in similar conditions.

Fraser, J. F.: Mycosis Fungoides, Its Relation to Leukemia and Lymphosarcoma. Arch. Dermat. and Syph., December, 1925, xii, No. 6, 814.

Fraser adds three cases to the growing number of instances in which mycosis fungoides appears to have been associated with neoplastic changes in the tissues.

In one case there was the development of a leucemic blood picture, in another the morphologic picture of lymphosarcoma. The third case was in a South American squirrel in which the lesions were identical with Sternberg's leucosarcoma.

The name reticulum cell sarcoma is suggested to replace lymphosarcoma in designating the lesions of mycosis fungoides on the assumption that the lesion has its origin in the reticulum cells of the papillary layer (reticulo-endothelial septum of the skin).

Roman, B., and Lapp, C. M.: Lesions of the Central Nervous System in Canine Distemper. Buffalo Gen. Hosp. Bull., September, 1925, iii, No. 2, p. 40.

The paper, which is excellently illustrated with numerous plates, reports the histologic examination of the brain and spinal cord in 29 cases of distemper.

The lesions, which were present in 19 animals, were in the main all similar and consisted of a definite inflammatory reaction of the pia mater in the region of the pons, medulla, and also in the cerebellum.

The infiltrating cells were mainly lymphocytic. Marked degenerative changes were found in the nerve cells, especially in the cord.

Stains for bactria were uniformly negative.

In brief, there was a disseminated polio- and leucomyelitis and areas of encephalitis associated with a patchy leptomenigitis with focal necrosis hemorrhages, and extensive degeneration of the nerve cells.

The authors believe that the work concerning the etiologic rôle of B. bronchisepticus requires revision.

Osgood, H.: Hemagglutinative Properties of Diphtheria Antitoxin. Buffalo Gen. Hosp. Bull., September, 1925, iii, No. 2, p. 37.

This report comprises the results of the examination of 26 specimens of stock antitoxin in which tests were made against the erythrocytes of a single individual for the presence of hemagglutinins in the antitoxin sera.

Agglutinins against human erythrocytes were found in all samples but this property, though relatively strong in fresh, unaged samples, did not appear to be strong enough in lots aged and issued for therapeutic use to cause trouble when intravenously injected.

Myers, C. N., and Cornwall, L. H.: Normal Arsenic and Its Significance from the Point of View of Legal Medicine. Am. Jour. Syph., October, 1925, xi, No. 3, p. 647.

As recalled by the authors, in an interesting introductory review, death by poisoning, either suicide or murder, and the use of arsenic as a lethal agent, are referred to in the earliest known medical literature.

Modern investigations have also led to the increasing use of this drug and its derivatives in the treatment of a variety of diseases. In as much as the chemical detection of arsenic in the body tissues is often of great forensic importance, it is essential to determine if its appearance is normal or entirely adventitious.

Prior to the work of Gautier in 1889 arsenic was regarded as an entirely adventitious substance in human tissues and so, upon occasion, of marked medicolegal significance.

Gautier reported its presence as a normal constituent of human tissues but these findings were disputed and disclaimed and have not been given much importance from a forensic

*Clinical Laboratory Medicine**

THE purpose of this book, as stated in the foreword, is "to present laboratory medicine through its clinical interpretation."

It is admittedly not intended for the research or advanced laboratory worker but for the student and practitioner.

The task which these authors have set themselves is one of some difficulty. So manifold are the resources of clinical pathology, and so hastily must they be reviewed in the crowded medical curriculum of the day that, it must be confessed, many students carry away the impression that it is the *test* which is of predominant importance rather than its *interpretation* and application to the patient.

Many overlook the fact that the division between clinical and laboratory medicine, so-called, is artificial and arbitrary; that the terms clinician and clinical pathologist should indicate, not two diverse and unrelated varieties of practitioner, but merely the particular phases of the medical art in which each has a compelling interest.

The first step in the management of disease is its recognition, its diagnosis, and to the formation of a diagnosis, the resources of the clinical laboratory may be so utilized as to bring much information that is of value and, sometimes, of essential importance. To apply this information and to utilize it to its full worth, the student and practitioner must realize that it constitutes only *a part* of the examination of the patient.

The manifestations of disease, in essence, are the manifestations of disturbances of function, the character and degree of which are many times best determined in the clinical laboratory. The ultimate *significance* of the abnormalities thus discovered depends upon the correlation of the findings thus obtained with all the other available data.

To present this subject in this way is a matter of some difficulty. If the treatise is comprehensive, it is apt to confuse the practitioner by the variety of methods and data presented; if too condensed, it fails, perhaps, to enlighten.

The authors have chosen a middle course.

The book appears to be essentially a manual of the methods taught to the students of the Long Island Medical College. In the main, the methods are well chosen and clearly described in detail and each is followed by some discussion of its clinical interpretation and application. At times this could be expanded with benefit.

There is an extensive section on blood chemistry embodying the technic of Folin and Wu and a very clear discussion of hydrogen ions and their determination. The laboratory criteria of acidosis are well presented. Clinical methods for blood transfusion, the therapeutic uses of convalescent serum in certain diseases, and the application of the Schick and Dick tests are discussed. The section on blood sugar does not note the practical papers of John on its clinical interpretation.

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*Clinical Laboratory Medicine, H. M. Feinblatt, M.D., and A. E. Eggerth, A.B., A.M. Pp. 411. Wm. Wood and Co., New York, $5.00.

The methods in general are modern. In the determination of red cell fragility, however, the relatively cumbersome technic of La Roche is described without mention of the rapid and simple method devised by Sanford and Giffin. The Wassermann technic described is that of Walker and Swift with a 0.4 per cent cholesterinized antigen and a primary incubation of one hour at 38° C. In view of the very extensive studies which have been conducted on this subject, no mention of which is made, this cannot be said to represent the latest consensus of serologists.

Under diacetic acid the Gerhard test is given without mention of the false reactions encountered when sodium bicarbonate is administered.

The proof reading and general preparation of the book are good and the illustrations apt.

References are placed directly in the body of the text "to facilitate rapid access." This innovation seems of dubious value. To the reviewer it seems to furnish an annoying break in the continuity of the text and an interference to smooth reading. It is also noted that the majority of the references cited seem to cover the older literature, few being later than 1919.

The book will serve a useful purpose for those to whom it is addressed.

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EDITORIALS

Clinical Electrocardiography and Recent Advances in Cardiac Physiology

CLINICAL electrocardiography, the laboratory method of precision par excellence, has come into its own well-merited and well-founded eminence through the painstaking studies of a long line of cardiac physiologists. Einthoven and Lewis have established firmly the foundation of modern cardiology. The clear conception of the mechanism of the heart beat in health and in disease has been made possible by the vigorous application of the method of the string galvanometer to the study of clinical cases of heart disease. Proficiency in bedside diagnosis and treatment of cardiac conditions is dependent upon experience that has been and continues to be frequently confirmed by electrocardiographic studies. The field of usefulness of the method is more and more generally appreciated. There are but few internists who do not realize the value of the method, in the detection and the determination of the exact type of conduction disturbances, such as a prolonged a-v. interval or a higher degree of block in the His bundle or of block in a branch of the bundle; in the recognition of auricular flutter and at times even of auricular fibrillation

with certainty; in the identification of the point of origin of a paroxysmal tachycardia; in the interpretation of murmurs and in changes or in a fixation of the electrical axis as evidences of asymmetrical hypertrophy or adhesive mediastinopericarditis; and, finally, in the gauging of the effects of therapeutic agents, especially drugs on the mechanism of the heart beat.

However, we have not arrived at this advanced stage of proficiency of the method at one jump. The rise has been by no means meteoric, and the progress, or advance henceforth will most probably be slow.

Our old theory of the development and propagation of the excitatory difference of potential and the resulting mechanical response by a rhythmic anabolic chemicophysical process in the specialized and ordinary cardiac tissues has been amplified and extended by the recent work of Andrus and Carter. These investigators have perfused the hearts of terrapins and dogs with isotonic solutions of known P_H value and have been able to "speed up" and to slow down, to stop and to start again the development of the excitatory process and its propagation by changing the P_H of the perfusion fluid. They conclude that the rhythm and rate of conduction in cardiac tissue depends to a great extent if not entirely upon the hydrogen-ion concentration of the blood and lymph. They consider the generation of the difference of potential (the negativity) to be due to a disturbance of ionic equilibrium between the cell contents, colloids and salts, sodium, calcium and potassium, on the inside of the semipermeable cell membrane and the surrounding tissue fluid of similar composition but of different concentration on the outside. Lillie's conception of the process of conduction depending upon the excitation of the adjacent area by the action current developed at the point of primary excitation is accepted to explain the propagation of the excitatory process in the heart, this in turn being determined by the difference of potential existing at the point of stimulation and by the permeability of the cell membrane, both of which circumstances depend upon the relative ionic concentration within and without the cell, which is finally in the last analysis also controlled by the hydrogen-ion concentrations. Disturbances in rhythm are thus the result of changes in P_H concentration not only in fluid, as Andrus and Carter point out, but in the tissues, the specialized and the ordinary heart muscle cells also.

Ashman in a recent important fundamental study compressed the auricular muscle and junctional tissues of the turtle heart, thus changing the P_H toward acid values in these tissues, and studied graphically the conductivity. He found (1) that following the transmission of an impulse through the compressed muscle there was at first an interval during which a second impulse if discharged from the auricle would fail to reach the ventricle; this was followed by a period during which the impulse was transmitted slowly, then more rapidly until finally the resting conductivity was regained. The course of the recovery was along a smooth curve. (2) Blocked impulses were found to influence the rapidity of conduction of subsequent impulses. The later the blocked impulse falls in the cycle, the more it prolongs the conduction time of the subsequent transmitted impulse. (3) Evidence suggesting slower recovery following blocked impulse was also given. (4) The interval of

the blocked impulse was considered by Ashman to include the earliest part of the relative refractory period.

Ashman in his second paper, also dealing with recovery of conductivity in compressed cardiac muscle, demonstrated a *supernormal* phase in conductivity. This phenomenon appeared under conditions which contributed to fatigue of the muscle, but fatigue effects were obtained without evidence of a supernormal, but never supernormal without fatigue effects. The recovery curves returning rapidly to a normal or resting conductivity passed beyond the base line for a greater or lesser distance and then gradually returned to the line representing the resting conductivity. Thus an impulse arriving at the compressed region four or five seconds after transmission of a previous impulse was actually transmitted more rapidly and in many cases much more rapidly than one delivered after ten, twenty or thirty seconds. With an increase in the degree of compression a stage might have been reached in which all impulses would be blocked excepting those falling on or near the crest of the supernormal phase. Lewis, and Wolferth have each reported a clinical case illustrating this phenomenon.

There was considerable difficulty, under the conditions of these experiments, in determining the exact form of the supernormal recovery curve. This was due to two phenomena. The first was the appearance, after rest, of a treppe or staircase in conductivity which caused the conduction times for a short series of impulses, each delivered during the supernormal phase left by its predecessor, to show a progressive decrease in length. The second was the appearance during continuous activity of cumulative fatigue, and the recovery from this fatigue which occurred after a minute or two. Ashman therefore obtained curves which showed, following the post-supernormal depression of conductivity, a gradual return of conductivity with prolonged rest. As a consequence in a typical case a degree of block might often be produced which would cause impulses arriving within 2.5 seconds following a previous impulse to be blocked; from 2.5 seconds to about 7 or 8 seconds, to be transmitted; from 8 to about 25 seconds, to be blocked; and after 25 seconds, to be transmitted. The success or failure of each individual impulse was also conditioned by the degree of cumulative fatigue or the antecedent appearance of a treppe in conductivity.

The treppe was best seen after an intermediate rest period, that is, before much recovery from fatigue had occurred. As a consequence of the treppe phenomenon when the compression was right and when a series of impulses at four second intervals arrived after a 20 second rest, the first impulse to be transmitted to the ventricle might be the third, fourth or even the fifth of the series. If the impulses continued to arrive at this rate several more may be transmitted in succession until a partial block developed as a result of fatigue. The block was ushered in by a prolongation of the conduction time. If, under these same conditions, a much longer rest was allowed before the series of impulses at four-second intervals was delivered at the compressed region, the first impulse might be transmitted most rapidly, the second almost as rapidly, the third somewhat more slowly, the fourth or fifth much more slowly until a partial block developed. Here the treppe was represented, not by a decrease

in the a-v. interval, but by the failure of the fatigue effects to appear as quickly as they otherwise would.

This relation is perhaps of importance for its bearing upon Iliescu and Sebastiani's criticism of the Kaufmann and Rothberger theory of parasystole (*Heart*, 1923, x, 101). Iliescu and Sebastiani state that the time relations of the transmission intervals postulated by Kaufmann and Rothberger are not those actually seen in partial block. If a supernormal phase were present, the time relations in question might have been possible.

It is fundamental work of this type that extends the bounds of our experience and blazes the way for further experimental and clinical investigation, that will add to the sum total of our knowledge.

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—*G. R. H.*

The Cleveland Meeting of the Federation of American Societies for Experimental Biology

THE annual meeting of the Federation of American Societies for Experimental Biology was held at Cleveland on December 28, 29 and 30, 1925. The yearly meetings of this organization may well be considered as the marking of milestones in the progress of American medical science. For, as a rule, these meetings really represent a summing up of practically all of the fundamental and basic discoveries made in biologic fields in this country during the preceding twelve months.

The federation is composed of four essentially independent societies, the Physiological Society, the Society of Biological Chemists, Inc., the Society for Pharmacology and Experimental Therapeutics and the Society for Experimental Pathology. In 1913 these independent societies formed the federation and they have met together each year since that date. It would perhaps be to the advantage of the federation if other scientific organizations such as the anatomists, the zoologists or the anesthetists might meet each year with the federation. This would undoubtedly result in a considerable financial gain to the attending members of the federation in those instances in which less than two hundred and fifty railroad tickets are sold to members visiting the meetings. It happened, however, this year for the first time, that the federation, meeting alone (*and in the west*), had in attendance a sufficient number of visiting members to require something more than three hundred railroad tickets and therefore the usual reduction in rates to one and a half fares for the round trip was allowed by the railroads. This small financial saving was undoubtedly of considerable importance to the very group of young men whom science most needs in this country at the present time. For it requires only a glance at the published programs of the federation to see that many of the new things presented, and, indeed, often discoveries of great and fundamental importance are brought forward by men whose names are new in the federa-

tion—men indeed whom only a year or so ago the members of the admissions committees were often weighing with judicial seriousness in the infallible scales of the past, the now and the hereafter to determine whether or not, if these men were admitted to the societies, they might lower the dignity of the organization. And, being human, it is probable that these committees have occasionally failed to read the scales correctly.

To the officers and faculty, and especially to the local committee of the medical school of the Western Reserve University are due the sincere thanks and the hearty praise of the whole federation for the splendid success of the entire series of meetings. The magnificent buildings and the splendid equipment of the new medical school undoubtedly served as a special attraction in inducing many members to attend the Cleveland meetings. A brief inspection of this splendid new group of buildings—a typical, modern, American medical plant—is sufficient to remind one again that all of the first-class medical schools in this country are rapidly approaching a fairly common standard so far as buildings, equipment, clinical material and, in the main, financial backing, are concerned. Wherefore we shall again revert to our former test for the standing and prominence of any given medical school. And the best school will again become the one that has the greatest number and the highest type of men on its faculty.

Ohio again touched a spot near to the hearts of the federation members, when, on Monday evening, December 28, the Western Reserve University tendered a splendid complimentary dinner to the members of the federation *and their wives* at the Mid-day Club. This is almost the first time that any group or organization in entertaining the entire federation has extended a special invitation to the wives of the members to attend any phase of the annual meetings, and the incident speaks most highly for the delightfully sympathetic and hospitable manner in which the university and the medical faculty put forth every effort to make the occasion a most enjoyable one for the whole federation. This will perhaps remind some of the older members of former times when the highest business ideal of American universities was to pay each man on the faculty a salary which was barely sufficient to support him (so long as he did not need the services of a doctor), and it was tacitly assumed among members of the societies that, as a matter of courtesy, no reference should be made to the possibility that some of the braver and more heroic members might be attempting to support wives in addition to themselves. It is a harbinger of the times that, in striking contrast to the old days, a goodly number of ladies, perhaps more than forty, attended the Cleveland meeting, and their names appeared on the programs either as sole authors, or as co-authors, of some thirty-six papers and demonstrations.

As mentioned above the federation is composed of four constituent societies. It is the custom that, in rotation through four yearly periods, the president and the secretary of each constituent society shall act as chairman and secretary respectively of the entire federation. In accordance with this rule Prof. A. J. Carlson of the University of Chicago and Prof. W. J. Meek of the University of Wisconsin served this year as general chairman and general secretary, respectively.

The first general session was held at the Hotel Statler on Monday morning, December 28. At this session the announcement of the deaths during the preceding year of Prof. A. W. Hewlett, of San Francisco, Prof. J. N. Langley, of Cambridge, England, Prof. Reynold A. Spaeth, of Baltimore, and Prof. Emeritus John Marshall, of Philadelphia, was made. As a mark of respect to the memory of these departed colleagues the members of the whole federation arose and stood with bowed heads for one minute.

This first session and also the final session on Wednesday afternoon were held at the Hotel Statler, an arrangement which proved exceedingly convenient from the standpoint of arrival and departure for the visiting members. All other sessions were held in the Western Reserve University Medical School buildings at 2109 Adelbert Road. A total of 209 papers were listed on the program to be read before the various societies. In addition to these 74 more were listed to be read by title. Most of Tuesday afternoon was given over to the presentation of demonstrations of which 30 were shown. The demonstrations constituted one of the most interesting and instructive features of the whole meeting, and each year more and more emphasis is placed on their development.

Dean Hamann of the Western Reserve University Medical School acted as toastmaster at the complimentary dinner given by the university on Monday evening. In a most deft and skilful manner (the dean is one of Cleveland's best known surgeons) the toastmaster opened the heart of the university in a cordial welcome to the federation. He introduced President Vinson who emphasized this welcome by stating that on a former occasion he had been placed as seventh man on a list of speakers all of whom had for their duty the welcoming of an organization which was then visiting Cleveland. The mere fact that President Vinson survived that experience is sufficient evidence to indicate that as second man only in welcoming the federation he did justice not only to himself and the federation but also added honor to his university. He further expressed the wish that scientific men might make themselves more aggressive and effective in combating the ignorance and the superstition which oppose modern scientific progress in general and the spirit of evolution in particular.

The next speaker presented by the toastmaster was Prof. Carlson who stated that it was only at the repeated, urgent request of the Cleveland local committee that he had at last agreed to prepare a speech for the Cleveland meeting. And when he began this task it appeared that he cast about for some desirable and inspiring subject, but the only really adequate and suitable material which he could find *in Chicago* was a series of jokes by Prof. A. S. Loevenhart of the Department of Pharmacology of the University of Wisconsin. These jokes, Prof. Carlson stated, he gathered up and "fumigated" (this may be the Chicago expression for *redistilled*) and finally condensed into an admirable speech. This incident indicates in a striking manner the very great dependence of present-day physiology upon pharmacology. After its completion the speech was mailed to the Cleveland committee for "expurgation," as Prof. Carlson had been warned that "Mayors, deans, university presidents, city managers, etc., all of whom could *talk*—whether they had anything to say or not"

month. Inasmuch as the A. S. C. P. meets during the
A. M. A. convention, there will be room for all our mem-
meeting, but unless reservations are made right now, the
for those who desire to stay over the ensuing week.

The Baker Hotel has been reserved as headquarters
This hotel offers ideal meeting rooms for our purpose.

Scientific Papers

Judging from the titles and abstracts of the papers to be
program will maintain the high level of excellence shown a
meetings. There is still an eleventh hour opportunity for our
have been dilatory in submitting titles of their papers to send
secretary at once for inclusion in the program. The subject m
articles submitted promises to be fruitful of discussion and of
profit to the clinical pathologist.

All Clinical Pathologists Are Invited

It has become a tradition with the A. S. C. P. to invite all clinic
ologists to our annual conventions be they members or not. To this en
five hundred invitations have been sent out to nonmembers extending to
the facilities of the Society.

The Society maintains a Service Bureau for its members. Any clin
pathologist wishing to make a change should communicate with the Secreta

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Kindly report any change of address to the Secretary.

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CLINICAL AND EXPERIMENTAL

METHODS OF STAINING TUBERCLE BACILLI*

BY H. J. CORPER, M.D., PH.D., DENVER, COLO.

IN choosing a laboratory test for practical purposes the clinical pathologist usually weighs speed, efficiency and reliability in his choice. There is no obvious reason why this policy should be deviated from in choosing a method for staining tubercle bacilli in suspected materials from human sources for diagnostic or prognostic purposes.

It is of historical interest that Koch[1] in 1882 stained the bacilli by means of methylene-blue (1 c.c. concentrated alcoholic in 200 c.c. distilled water + 0.2 c.c. of 10 per cent potassium hydroxide), using freshly filtered concentrated watery vesuvin solution as a counterstain, thus obtaining blue bacilli on a brown field, while Baumgarten[2] at the same time studied them unstained by contrast in a stained field. Koch early recognized the resistance of the bacilli to staining as well as destaining and utilized a small amount of potassium hydroxide in the methylene-blue stain for this reason to aid penetration. Originally he used no decolorizing agent but after staining for long periods at room temperature, or shorter periods with warming, applied the counterstain vesuvin. The shortcomings of the Koch method were early recognized and Ehrlich[3] introduced aniline oil into the staining technic with decided improvement. The Ehrlich method consisted essentially in staining the smear or section (twelve hours cold or less time warm) in a solution containing 11 c.c. of concentrated alcoholic methyl-violet or fuchsin in 100 c.c. of saturated aniline oil water and 10 c.c. absolute alcohol, decolorizing in dilute (1:3) nitric acid for a few seconds, rinsing in 60 per cent alcohol and counterstaining in dilute

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vesuvin. It is interesting to note that Ehrlich stained the bacilli with a large number of basic stains including vesuvin or Bismarck brown.

There rapidly followed an elaborate literature and numerous new methods for staining tubercle bacilli, each claiming advantages and adding valuable information to the sum total knowledge on this subject. Of practical importance, however, were the introduction of carbolic acid solution of the basic stains by Ziehl[4] and the use of carbol diamant-fuchsin by Neelsen[5] constituting the rapid Ziehl-Neelsen method now so widely used routinely for staining tubercle bacilli.

In order to still further simplify the technic Gabbett,[6] on the basis of a recommendation made by B. Fränkel earlier, combined the decolorizing and counterstaining by using a 25 per cent watery solution of sulphuric acid containing 1 to 2 grams of methylene-blue in 100 c.c. of solution. From the time Koch found potassium hydroxide aided penetration of the bacilli by the stain and Ehrlich used aniline oil for this purpose there resulted a large number of methods based on the use of different mordants including, besides carbolic acid, resorcin, pyrogallic acid, ammonium carbonate, ammonium hydroxide, boric acid, chloroform, creosote, menthol, mercuric chloride, and others. So, likewise, there have been recommended a large list of decolorizing agents including acetic, hydrochloric, nitric and sulphuric acids, acid alcohol, lactic acid, acetone, sodium sulphite, hydrogen peroxide, calcium hydrochlorite and even hot water.

Since it was practically impossible to test out all the methods that have been recommended for staining tubercle bacilli, only those will be referred to here which were considered of sufficient importance to be included in a comparative test when this study was initiated. They were chosen for a number of reasons among which were that they were recommended from a number of sources in the literature; the method served basically for the origin of a number of later methods and it incorporated essentially a new principle founded on seeming sound ideas.

Among the older methods in which a chemical mordant was emphasized was that described by Herman[7] who used for this purpose ammonium carbonate. The stain consists of 3 parts of 1 per cent ammonium carbonate solution in distilled water and 1 part of 3 per cent crystal violet in 95 per cent methyl alcohol. The smear or section is stained for a few minutes steaming and is then decolorized with 5 or 10 per cent nitric acid solution for a few seconds after which it is dipped a few times in 95 per cent alcohol and then is rinsed in tap water and is counterstained with 1 per cent eosin solution (originally he used 60 per cent alcohol as solvent). The bacilli appear violet in a rose field. In 1908 the author claimed to find bacilli when Ziehl-Neelsen's method failed and that it was superior to Much's method.

Another of the earlier methods was that of Andrejew[8] who not only utilized the mordanting idea, using for this purpose potassium chloride, but laid stress upon complementary counterstaining. It is pointed out that green is the most suitable counterstain for red bacilli and of the available green stains he prefers acid green to malachite green. The preparations are stained by the ordinary carbol-fuchsin solution and are decolorized and counterstained

for about one minute in a mixture of (1) hot 10 per cent potassium chloride solution 100 c.c.; (2) acid green 1 gram; and (3) 25 per cent sulphuric acid 15 c.c.; the mixture having been well shaken and filtered.

Among the serviceable yet uniquely developed methods for staining tubercle bacilli may be included that described by C. Spengler,[9] and devised to fit the theory that tuberculosis in man usually was a double infection with human and bovine bacilli which could be differentiated by his staining methods. He also believed that the presence of granules, called by him "Splitter" or "sporoide Körper" demonstrated by one of the three methods devised by him, was of diagnostic value. Later he considered them spores, analogous to the spores of anthrax. The picrin method of Spengler essentially consists of staining with warm carbolfuchsin for five minutes, which is then poured off, and picric acid-alcohol (50 c.c. saturated watery picric acid + 50 c.c. absolute alcohol) applied for two to three seconds, followed by three to four drops of 15 per cent nitric acid and then by picric acid 5 to 10 seconds until the sputum takes on a faint yellow color after which the smear is rinsed in water and dried; or after picric acid treatment the smear is rinsed with 60 per cent alcohol followed by 15 per cent nitric acid (a few seconds) until the sputum assumes a light yellow color, when it is rinsed with alcohol, followed by counterstaining with picric acid alcohol to a yellow color, after which the smear is rinsed in water, dried and examined. It is claimed that the method gives positive results when the Ziehl-Neelsen and other methods fail and that it is specific for acid-fast organisms. The light red bacilli and isolated "splitter" are distinctly seen in the light yellow background.

Until Much[10] emphasized the importance of Gram staining the granules of tubercle bacilli the attention had been focused mainly on the importance of mordanting. Much's method consisted of 3 distinct technics all based on the use of iodides such as are used in making the ordinary Gram stain.

Much's technics were as follows:

Gram method I.

1. Aniline water-gentian violet;
2. Lugol solution;
3. Decolorizing in absolute alcohol and clove oil.

Gram method II.

1. Methyl-violet B. N. 10 c.c. saturated alcoholic solution in 100 c.c. 2 per cent carbolic acid water. Steam over the flame three minutes or warm twenty-four to forty-eight hours at 37° C.
2. Iodpotassium iodide one to five minutes;
3. Five per cent nitric acid one minute;
4. Three per cent hydrochloric acid ten seconds;
5. Acetone-alcohol āā.

Gram method III.

1. Methyl-violet B. N. solution as above;
2. Potassium iodide-hydrogen peroxide solution (5 g. KI in 100 c.c. 2 per cent H_2O_2) for two minutes;
3. Absolute alcohol.

Much claimed that his method would reveal all the granular forms of tubercle bacilli not stained by the Ziehl-Neelsen's method and concludes that it seems to him that the Ziehl method stains substances in the tuberculosis virus which differs from that stained by Gram, and that the Gram method still gives

positive results when the Ziehl staining substances are lacking. In a polemic with Fuchs-Wolfring, Much[10a] also takes a definite stand regarding the relation of the Gram granules and Spengler's "Splitter." He says "the Much granules are not acid-fast. The Splitter are acid-fast (picrin method)."

Rapidly following this observation by Much there were reported a number of combination stains developed in an endeavor to combine the advantages of Gram staining and bacillary staining based upon either the Spengler or Ziehl-Neelsen methods. One of these was described by Fontes[11] who also believed his method would differentiate the true tubercle bacillus from pseudotubercle bacilli. The smears are stained in carbolfuchsin for one minute steaming and are then allowed to stand in contact with the stain two minutes longer when they are rinsed with water and stained for two minutes with steaming crystal violet or carbol gentian-violet after which the excess stain is removed and Lugol's solution allowed to act on the smear until no further metal mirror forms. The preparation is decolorized in acetone-alcohol (āā), is washed in water and counterstained with methylene-blue. It is claimed that the acetone-alcohol frees all the pseudotubercle bacilli from fuchsin. The violet granules appear very contrasty in the red bacillary bodies. Pseudotubercle bacilli are violet and have deeper violet granules while other gram-positive microorganisms (streptococci, staphylococci, etc.) are gram-positive and all other bacteria are blue. If the staining with fuchsin and crystal violet is reversed the same results attain but the picture is less clear. The granules vary from 1 to 6 in each bacillus although rarely there may be 8 to 10. Fontes concludes the granular forms are the more resistant forms.

For the purpose of differentiating of smegma from tubercle bacilli in the urine Gasis[12] has taken advantage of the alkali fastness of the tubercle bacillus in devising a method. "Tubercle bacilli behave amphoterically to acids and alkalies. Smegma bacilli are partly acid and alcohol resistant. Smegma bacilli are not alkali resistant. Tubercle bacilli are partly acid and alcohol resistant. Tubercle bacilli are alkali resistant." The Gasis method based on the foregoing facts consists of staining the fixed preparation one to two minutes warm with the dye-mordant combination made by boiling a small lentil size lump of mercuric chloride in a few (5) cubic centimeters of 1 per cent eosin solution (1 g. eosin, 5 c.c. absolute alcohol and 95 c.c. distilled water), with constant shaking until the mercuric chloride is completely dissolved. The smear is then rinsed in water and decolorized by a solution consisting of 0.5 sodium hydroxide, 1.0 potassium iodide and 100 of 50 per cent alcohol, until the red color has disappeared and a white green color develops. The decolorizing solution is removed by means of absolute alcohol and the smear is rinsed in water and counterstained with methylene-blue (1.0 g. + 10 c.c. absolute alcohol + ½ c.c. hydrochloric acid + 90 c.c. distilled water) for 2 to 3 seconds after which it is rinsed in water and dried for examination. The bacilli are a beautiful light red in a blue field. Gasis believes the granules obtained with this method are not identical with those obtained by the Much-Gram stain. The Gasis granules are deeper stained and more resistant than the bacilli he claims.

Within recent years there have been described three new modifications of the older methods which seem to merit consideration. One of these devised by

Konrich in 1920[13] may be attributed directly to the economy of war conditions. When the price of alcohol soared in Europe Konrich remembered the decolorizing reducing action of sodium sulphite on fuchsin in the preparation of Endo's medium and believed this might be advantageously and economically substituted for decolorizing in the Ziehl-Neelsen method for staining tubercle bacilli. The fixed preparation is stained one-half to one minute with hot carbol fuchsin, rinsed in water and is then treated a few seconds with a fresh 10 per cent watery solution of sodium sulphite after which it is rinsed in water and counterstained with malachite green solution (50 c.c. saturated watery solution + 100 c.c. water). He claims that acid alcohol may decolorize the bacilli but sodium sulphite does not, since it cannot enter the bacilli to reduce the fuchsin in them. They remain stained even after 24 hours treatment with sodium sulphite. Malachite green is preferred on account of its color contrast to the red bacilli and because it does not stain the rest of the cellular elements too deeply.

Another more recent modification was proposed by Burke and Dunning[14] who incidentally included the staining of the tubercle bacillus with the method for staining spores. The method essentially is apparently one of making a combined carbolfuchsin-Gram stain of the bacilli even though it is stated that "carbolfuchsin under the most favorable conditions does not give a good Gram reaction." The smear is stained in steaming carbolfuchsin for two minutes or longer, is washed in water, and decolorized with acetone or iodine acetone (1 part Gram's iodine solution to 3 parts acetone, or a few crystals of iodine is added to acetone) until the smear is nearly colorless which requires a few seconds, and is counterstained with 10 per cent aqueous solution of a saturated alcoholic solution of methylene-blue for one to two minutes or a saturated aqueous solution of picric acid for from five to ten seconds. Smears of tuberculous sputum stained by this method showed as many acid-fast organisms as when stained by the Ziehl-Neelsen method.

The third of the more recent methods by Serkowski[15] utilizes the fact frequently referred to as an advantage of the Spengler method—the possibility of staining the bacilli in thick smears without material interference with their visibility. The method may be considered a combination staining method in that 2 dyes, carbolfuchsin and carbol-methyl violet, are used with sodium carbonate as the mordant. (1) Liquid sputum is laid on a slide in several consecutive layers, each layer being allowed to dry before applying the next one. As the background remains practically unstained by this method, the thickness will not mask the bacilli; (2) cover film with carbolfuchsin without previous fixation and hold over flame two minutes during which steam must appear several times. (3) Without washing the film a 1 per cent solution of crystallized sodium carbonate is applied which is boiled over a flame two minutes, during which it may be necessary to add fresh sodium carbonate solution. The film must not be washed in water, but the excess of sodium carbonate is simply shaken off. (4) Stain with one part carbolfuchsin and three parts of carbol-methyl-violet (ten parts saturated alcoholic solution of methyl-violet and ninety parts 2 per cent carbolic acid in water) over flame for two minutes, steaming without ebullition. (5) Shake off excess stain, without washing in water, and

treat five minutes with 10 per cent solution of sodium sulphite (Konrich) adding a few drops of 60 per cent alcohol during the last stages of the decolorizing procedure. (6) Without washing, counterstain with 1 per cent picric acid for thirty seconds. (7) Remove all stain spots from the edges and back of slide with a cotton wool swab saturated in alcohol and acetic acid. (8) Then only is the slide dipped once or twice in still water and dried in air.

Serkowski claims this method increases the number of bacilli on the films 50 to 60 times over the Ziehl-Neelsen method and believes homogenization methods make the cortices of the bacilli less resistant to staining. Thistlethwaite[16] used this method and found on an average 8 bacilli to 1 in Ziehl-Neelsen preparations. She uses two or three layers of sputum fixed by heat before staining and prefers 10 per cent sulphuric acid for decolorizing.

In an attempt to accentuate the staining of tubercle bacilli Biot[17] after staining with carbolfuchsin and decolorizing with nitric acid places the preparation in commercial formalin for two to four minutes when, it is stated, the bacilli stand out as black rods. No counterstain is necessary by this method.

Fat stains have never been used with success for staining tubercle bacilli even though Dorset[18] at one time recommended certain of them (Sudan III and Scarlet R) for this purpose.

The opinions expressed and conclusions drawn from a study of a number of different staining methods for tubercle bacilli by some of the foremost students of this subject is worthy of brief mention here. Joest[19] in 1908-9 states as a result of experiences with the Much and Ziehl-Neelsen staining methods on tuberculous material that "the Gram (Much) staining for the bacterioscopic determination of the tuberculosis virus in lesions of spontaneous tuberculosis in cattle and pigs possesses no advantages over the Ziehl staining." Eisenberg[20] expresses himself in agreement with Liebermeister that in so far as diagnostic significance is concerned only rows of granules, and then only in otherwise bacterial free materials, are of value; dust like or free granules even though interesting theoretically are too little elucidated and too manifold in origin to be of any serious weight for diagnosis in practice.

As a result of extensive study with a large variety of tuberculous materials from animals and man using the Ziehl-Neelsen, Much and Gasis methods of staining tubercle bacilli Berger[21] in 1910 concluded that the Ziehl-Neelsen method is still the most reliable method, being negative only in a small number of cases positive by other methods, and it has the advantage of being rapidly and easily performed. It is especially reliable in cases of mixed infection where the Gram stain colors other rods. The disadvantage is that the granular forms are not stained by this method. The preparations by this method are also not permanent—the bacilli gradually becoming paler. The Much modified Gram method stains isolated and rows of granules, homogeneously stained rods are only rarely seen. This method is of great significance because it stains the granular forms of tubercle bacilli. The disadvantages of this method are the dangers of error in mixed infections and the entire picture is not as clear as after Ziehl-Neelsen staining. For rapid staining and speedy location of the bacilli the Gram method is less suitable than the Ziehl-Neelsen. It is not suited for permanent preparations. The Gasis method is especially suited for

structural studies of the tubercle bacillus. The disadvantages in comparison to the Ziehl-Neelsen method are its complicated nature and the greater percentage of failures in staining.

In an inaugural dissertation presented in 1910 Adam[22] tested a number of methods [Ziehl, Spengler, Gram (Much), Gasis, Berka, Knoll (a combination stain) and others] on a variety of tuberculous materials from man, cows, pigs, horses and chickens, and concludes that the picrin method of Spengler is of equal value to the Ziehl method. It has advantages over the Ziehl method when methylene-blue is used in the latter as counterstain. The Herman method, especially the Berka modification, is recommended as an adjunct to the Ziehl method. The Gram method of Much reveals a special form of tubercle bacillus not visible by the Ziehl method, but this method alone is not sufficient to certify a diagnosis of tuberculosis. The method of Knoll beautifully reveals the Much and Ziehl forms of tubercle bacilli but is only suited for a study of the finer structures of the tubercle bacillus. The method of Gasis is useful in differentiating tubercle bacilli from other acid-fast rods and for structural studies, but cannot replace the Ziehl method. None of the staining methods studied served to differentiate the types of bacilli.

In another inaugural dissertation presented in 1911 Barnowsky[23] tested sputum and other tuberculous material from man and animals, with and without antiformin treatment, by staining it according to the Ziehl-Neelsen and Much staining technics. He concludes that the Ziehl method stains tubercle bacilli homogeneously, rarely granular, while the Much method stains them granular, but rarely homogeneously. The granules occur in rod forms, occasionally in dust form. For diagnosis only the rod forms not the isolated granules can be evaluated since the latter have no definite form or size, and therefore cannot be differentiated from cocci, dye precipitates and other contaminations. Both methods fail to give permanent preparations; Much preparations last only twenty-four hours. Much's method after antiformin treatment must be cautiously interpreted since Gram positive rods and cocci can be mistaken for tubercle bacilli. Since the Ziehl technic is much more rapid and simpler than the Much technic, the former is given the preference. In suspected cases the Much technic can be utilized but in such cases animal inoculation must be relied upon to avoid mistakes in diagnosis.

Dold[24] in reporting on the differential diagnostic significance of various staining methods in 1911 states that there is no specific staining method available for the differentiation of the genuine tubercle bacillus from nonpathogenic acid-fast organisms and that comparative tests of the Ziehl and Gram (Much) methods on tuberculous tissues (lymph glands and spleen of guinea pigs) revealed no significant difference in the number of bacilli by these two methods. He does not wish to dispute the existence of non-Ziehl or non-acid-fast staining forms of tubercle bacilli but believes the diagnostic value of Gram positive rods or granules is slight. The double staining methods give instructive pictures and the Herman method is simple and serviceable. In 1911 Böhm[25] reported on trying 24 different staining methods for tubercle bacilli and concludes that "In the examination of suspected sputums the best results can be obtained with the Ziehl-Neelsen method since a bacillus found by this method

certifies the diagnosis. If the Much method is used and only granules are found a definite diagnosis cannot be made. Other methods do not come into consideration since they are partly less reliable and partly more complicated than the Ziehl method. On a par with the Ziehl method may be included the Ehrlich, Spengler and Herman methods."

In a recent article Geschke[26] compares the Spengler method and one of its modifications, the Jötten-Haarmann, with the Ziehl-Neelsen method and two of its recent modifications, the Konrich and Schulte-Tigges methods. Of these five methods the Ziehl-Neelsen and Konrich with blue and green background proved the more pleasing to the eye but the bacilli are more distinct with the yellow counterstain. The Spengler and Jötten-Haarmann stained preparations of 56 sputums studied revealed far more bacilli than by the three other methods.

The study to be reported here was initiated for the purpose of helping elucidate the practical problem of staining tubercle bacilli for diagnostic purposes, and in addition to note where discrepancies in the practical methods were occurring with a view to correcting these and if necessary instituting a more standard and reliable technic than at present available. In order to obtain as much information as possible on the various better known staining methods for tubercle bacilli and especially those having initiated a new staining principle, 20 sputums positive for tubercle bacilli were stained by twelve different technics incorporating in some cases the ordinary principles of staining with a mordant as exemplified by the Ziehl-Neelsen method, in others the Splengler principle, and in some the Gram (Much) principle, or a combination of the latter with one of the former two. The methods used were the original Koch methylene blue-vesuvin method, Ehrlich's aniline oil-gentian violet method, Ziehl-Neelsen's carbolfuchsin-methylene blue method, Andrejew's fuchsin-acid green method, Spengler's carbolfuchsin-picric acid method, Much's methyl-violet B.N.—Gram method, Gasis' eosin mercuric chloride-methylene blue method, Herman's crystal violet-eosin method, Fontes' combination carbolfuchsin-Gram method, Konrich's modified carbolfuchsin-sodium sulphite method, Burke and Dunning's carbolfuchsin iodine method, and Serkowski's combination carbolfuchsin-carbolmethylviolet method. About three months were spent in trying out the various above methods in preparation for the staining of the twenty sputums so that all the methods might be given an equal chance in the final test on the sputums. In order to make the different smears from the same sputum as nearly alike as possible the sputum was well shaken with glass beads, filtered through cheesecloth, a portion thus well mixed placed on one glass micro-slide and two smears made by placing another slide on this one and drawing them apart. Every precaution was taken to have the smears from the same sputum alike in thickness and uniformity. Any that appeared to deviate from this even in general appearance was discarded. After the usual heat fixation all the smears of the 20 sputums to be stained by a certain method were stained at the same time and with as nearly as possible the same technic. After staining they were examined and the bacilli counted in an average of 100 high power oil immersion fields using a mechanical stage to obviate counting the same field twice and to permit a systematic examina-

tion of the smear. The results in number of bacilli found in the average field for each preparation are given for ten sputums only since they suffice to illustrate the significant findings. The results are given in Table I.

TABLE I

THE RESULTS OF THE EXAMINATION OF POSITIVE SPUTUMS FOR TUBERCLE BACILLI STAINED BY DIFFERENT TECHNICS

STAINING METHOD*	SPUTUMS									
	1	2	3	4	5	6	7	8	9	10
Koch	–	–	3.4	0.17	–	5.9	–	1.2	–	2.2
Ehrlich	3.2**	8.5	5.3	7.5	6.8	17.2	11.0	–	4.6	7.3
Ziehl-Neelsen	3.1	8.2	17.9	10.9	10.8	15.0	4.1	1.3	2.7	8.2
Andrejew	2.9	9.1	7.8	10.0	11.2	14.1	2.5	–	0.5	6.3
Spengler	3.2	10.0	3.0	7.0	8.1	14.1	6.1	1.5	1.7	5.0
Much	3.5	8.3	6.2	5.1	6.7	11.3	5.8	2.0	2.6	3.7
Gasis	–	4.8	5.9	–	2.4	–	4.4	–	–	5.2
Herman	3.3	14.7	14.0	7.5	4.6	4.87	3.1	–	0.4	10.0
Fontes	3.0	9.5	4.1	6.7	2.5	16.9	4.5	2.6	1.3	3.5
Konrich	3.5	15.6	14.2	12.3	15.3	20.0	10.2	1.5	2.2	4.9
Burke & Dunning	3.1	22.2	10 6	12.2	9.4	12.2	–	2.1	–	7.0
Serkowski	–	12.8	4.7	15.3	9.5	6.1	4.1	0.8	1.8	3.8

*Biot's method is not included in the tabulation although the sputums were stained by this method and examined, since it is only an intensified carbolfuchsin method which is more difficult of examination because of lack of counterstain, and the method seems to gain nothing at the expense of more cumbersome technic.

**The numerals indicate the number of bacilli per field obtained as an average from the examination of about 100 different fields. A dash indicates no bacilli were found stained in the preparation.

An examination of Table I reveals two outstanding points; first, that in spite of the fact that every precaution was used in staining the preparations, there were some smears in which the bacilli were not stained and this was not confined to any one technic but occurred irregularly with different methods, in addition to which there were at times obtained low counts seemingly attributable only to a poorer staining and, second, in spite of the precautions taken to obtain uniform smears these varied to the extent of permitting a difference of 100 per cent in the counts of individual smears. Some staining technics proved more difficult of performance as is evidenced by the greater number of failures. Among these were Koch's original method, Gasis and Biot's methods. A few methods proved unsatisfactory because of unsuitable staining of the bacilli, lack of a background counterstain or lack of contrast between the bacilli and remainder of the smear. Among these were Biot's and Serkowski's methods. Much's method proved entirely unsatisfactory with sputum on account of the difficulty encountered in differentiating the tubercle bacilli from other small cocci since only the beads or granules in the majority of cases stained by this method.

In order to exclude the variations consequent to the making of different smears from the same sputum, 16 different positive sputum smears were consecutively stained, decolorized and restained by nine different methods—Ziehl-Neelsen, Andrejew, Herman, Konrich, Gasis, Fontes, Burke and Dunning, Ehrlich, Spengler, and finally Ziehl-Neelsen again. The tubercle bacilli in an average of about 100 fields were counted on each smear. The findings for ten of sixteen of these sputum smears are recorded in Table II.

TABLE II

THE CONSECUTIVE RESTAINING OF POSITIVE SPUTUM PREPARATIONS FOR TUBERCLE BACILLI BY DIFFERENT STAINING TECHNICS

STAINING TECHNICS	SPUTUMS									
	1	2	3	4	5	6	7	8	9	10
Ziehl-Neelsen	6.2*	9.1	7.5	34.0	22.3	9.6	17.7	5.2	31.3	9.7
Andrejew	4.6	9.2	7.7	39.2	-	7.3	18.4	5.4	26.5	-
Herman	4.9	10.7	6.6	28.1	11.5	10.6	20.5	7.6	27.8	10.9
Konrich	3.4	10.7	4.4	28.2	32.2	11.2	23.4	6.9	24.6	11.0
Gasis	1.3	4.5	1.5	8.6	-	12.7	19.8	6.0	10.4	2.1
Fontes	4.9	7.6	7.0	25.3	19.7	8.0	19.0	5.0	25.9	8.9
Burke & Dunning	4.5	9.3	3.3	26.6	16.9	10.9	19.0	6.7	28.3	12.3
Ehrlich	3.3	11.8	4.7	23.1	10.2	9.6	14.5	5.0	25.3	10.6
Spengler	3.2	3.2	-	26.4	15.2	10.4	15.3	4.6	21.2	5.8
Ziehl-Neelsen	5.8	10.5	6.9	24.1	23.1	10.1	23.6	6.8	37.6	9.7

*The numeral indicates the bacilli in an average field of about 100 counted; a dash indicating no stained bacilli were found.

Table II brings out clearly the importance of technic in the practical staining of tubercle bacilli rather than the importance of method which seems to have been emphasized mostly in the past. The choice of method resolves itself more into the question of getting thoroughly acquainted with one or two of them that happens to meet the fancy of the operator and by performing them accurately and carefully they will give results approximately equal to any of the other reliable methods. Of course, sight must not be lost of the fact that, given the choice of two equally efficient methods, the time element remains the important factor. This table also gives a good insight into the possible variations in counts resulting from slight possible deviations in technic. It will be noted in addition that a combination, double bacillary stain technic as exemplified in the Fontes method, neither increased the efficiency of the simple single bacillary stain methods nor did it obviate the deviations, consequent to slight variations in technic, of the single bacillary stain method.

In an effort to obtain more information on the granules staining by the carbolfuchsin simple mordant technic as exemplified in the Ziehl-Neelsen method, four positive sputum slides were stained gradually by means of steaming carbolfuchsin, each time being examined after decolorizing in 3 per cent acid alcohol and counterstaining with methylene-blue, and it was noted that in all four slides with faint bacillary staining there was a distinct granule staining present in the large majority of the tubercle bacilli in the smear. The granules were distinctly red when the bacillary body was a faint pink or was still unstained. As the stain intensity increased the granules became a dark red in contrast to the lighter red of the bacilli and finally with additional staining the entire bacilli assumed a dark purplish red uniformly stained appearance. Comparison of these carbolfuchsin stained slides during the predominant distinct coloring of the granules revealed them in numbers from 1 to as high as 6 or 8 in the individual tubercle bacilli and except for the deep red color of the granules as contrasted to the dark blue of the Gram (Fontes and Much) stained slides of the same sputums they seemed to be identical. The above four smears were then gradually decolorized by means of 3 per cent acid alcohol and after counterstaining with methylene-blue were repeatedly examined. Gradually the tubercle bacilli lost their intense dark reddish color

and the dark granules again appeared in a lighter red color of the bacilli themselves. As the decolorization proceeded both the granules and bacilli became lighter red but the granules were regularly darker than the remainder of the bacilli and finally there were in certain instances found granules still stained red with the rest of the bacillary body unstained and only seen as a transparent colorless shadow barely visible only in a few cases. These same four slides were then stained by the Gram and carbolfuchsin combined method and only the blue Gram granules could be found, there being no red granules, which fact would seem to speak in favor of the identity of the two types of granules. If the Gram granules and ordinary carbolfuchsin staining granules obtained during Ziehl-Neelsen staining differed it would seem that the combination stains at times at least might reveal both red and blue granules in a pale pink tubercle bacillus. It must be admitted that the blue Gram granules in the red bacillus are much more striking to the eye than the red granules but this concerns mainly the more striking color contrast of blue on red as compared to dark red on red or red on pink which also tends to make the granules look different both in size and morphology. The contention that the Gram granules are not acid-fast seems incorrect also, since Gram (Much) stained preparations may cautiously be stained with steaming carbolfuchsin and decolorized with 3 per cent acid alcohol removing the fuchsin from all parts of the smear except the bacilli, and blue granules are still visible in the red bacilli.

Thus far sputums containing besides tubercle bacilli numerous other microorganisms, were used as tests of the various staining methods and since the Gram modifications are supposed to possess advantages in the examination of pus and materials free from other microorganisms, a series of 12 samples of pus were stained by the Ziehl-Neelsen, Spengler, Much and Fontes methods, 100 fields being counted in each specimen and the findings recorded in Table III. The pus examined was obtained from guinea pigs inoculated subcutaneously, thirty to thirty-five days prior to obtaining the specimen, with avirulent ("Human") and virulent ("Gluckson") human and virulent ("Bov. Vir.") bovine tubercle bacilli. The pus was well ground up, first as obtained and then after the gradual drop by drop addition of sterile distilled water, until a fine

TABLE III

RESULTS OF THE EXAMINATION OF PUS, CONTAINING HUMAN OR BOVINE TUBERCLE BACILLI, STAINED BY THE CARBOLFUCHSIN (ZIEHL-NEELSEN AND SPENGLER) AND GRAM (MUCH AND FONTES) METHODS

STAINING METHOD	AMOUNT IN MILLIGRAMS AND TYPE OF BACILLI USED FOR INFECTING GUINEA PIGS SUBCUTANEOUSLY											
	"HUMAN" (AVIRULENT HUMAN BACILLI)				"GLUCKSON" (VIRULENT HUMAN BACILLI)				"BOV. VIR." (VIRULENT BOVINE BACILLI)			
	50 MG.	50 MG.	1.0 MG.	1.0 MG.	0.1 MG.	0.1 MG.	0.001 MG.	0.001 MG.	0.1 MG.	0.1 MG.	0.001 MG.	0.001 MG.
Ziehl-Neelsen	0.23*	0	0	0	0.17	0.19	0.09	0.02	0.18	0.12	0.27	0.05
Spengler	0.16	0	0.01	0.18	0.11	0.09	0.08	0	0.09	0.05	0.12	0.02
Much	0.06	0	0.01	0	0.19	0.02	0.03	0	0.10	0.03	0.15	0
Fontes	0.02	0	0	0	0	0.01	0	0	0.04	0	0.05	0

*At least 100 fields were examined in each preparation and the numeral given is the average number of bacilli per field deduced therefrom. The staining and counting of bacilli was performed by different individuals. The counting was checked by two individuals to obviate as far as possible individual error and in the Gram stains only bacillary forms were counted to avoid the possibility of including individual cocci or dye precipitates as tubercle bacilli. Any semblance of bacillus was, however, counted as such.

uniform suspension resulted. The smears were made as uniformly as possible and after heat fixation were stained.

An examination of Table III reveals no decided advantages of the Gram over the ordinary carbolfuchsin methods although it must be admitted, even though the reverse appears evident from the table, that as a method, except for more complicated technic involved, the combined method (Fontes) properly performed probably gives equally good results to the simple carbolfuchsin methods. The simple Gram Much technic is always fraught with the danger of misinterpretation for practical purposes. The variable figures recorded in Table III again emphasize the difficulty of making absolutely uniform smear preparations and the importance of proper staining technic. It is for this reason that it seems advisable especially that the clinical pathologist, who is generally called upon to examine different materials of which he can frequently obtain only a single sample and who has manifold methods for different diagnostic purposes with which he must keep efficiently conversant, have available a technic that will rule out or reduce it to a minimum. In the tuberculosis sanatorium or hospital where repeated specimens can be obtained this may not be so essential but even there it would seem advisable in examining certain specimens such as urine, pus and spinal or pleural fluid. It is a well-known fact that tubercle bacilli retain their staining properties for years and that even boiling or autoclaving and certain drastic chemical treatments will not materially affect this. It is an easy matter, therefore, for every clinical pathologist to keep at hand a standard suspension of tubercle bacilli which he can appropriately mix with a sample of the specimen to be tested—urine sediment, spinal fluid, pus, etc.—and making a control smear with this can carry it through the identical technic as that used on the test smear. This will give him confidence in his technic applied to the particular material at hand being tested. If his technic is correct the bacilli in the control preparation should stand out well stained and in number equivalent to his accustomed observations with the same suspension. On account of the difference of staining and destaining of thick and thin portions of smears it also seems advisable to make control and test smears as nearly uniform as possible.

In tests of various mixtures for this purpose it has been found that a suspension of tubercle bacilli made by carefully grinding tubercle bacilli with 0.9 per cent sodium chloride solution and finally diluted to contain 1 to 10 milligrams of tubercle bacilli per cubic centimeter in 50 per cent acetone-saline solution, mixed with sputum, pus or urine in proportion of approximately 0.1 c.c. of suspension to 0.9 c.c. of material to be tested, gives sufficient bacilli (5 to 10) in the ordinary oil immersion field to yield a speedily examined check on the staining technic. The acetone-saline suspension of tubercle bacilli keeps well in a rubber-stoppered bottle and on shaking gives a good uniform suspension each time. Suspensions made with eggwhite, gum acacia and glycerol did not prove satisfactory. The one danger in the use of this—control method—is that of contamination of the test specimen under examination, which would lead to erroneous positive findings. The careful worker need

not be warned of this danger, yet it must be kept in mind where the work of testing specimens is relegated to careless or untrained technicians.*

SUMMARY AND CONCLUSIONS

An examination of 20 sputum smears containing tubercle bacilli by thirteen different staining methods (Koch, Ehrlich, Ziehl-Neelsen, Andrejew, Spengler, Much, Gasis, Herman, Fontes, Konrich, Burke and Dunning, Serkowski, and Biot) revealed that when the technic is properly performed the majority of these methods may result in staining the tubercle bacilli in the sputums with equal efficiency. There was, however, a decided difference in the ease of performance, speed and simplicity of the various individual methods. Sixteen different positive sputum smears consecutively stained and restained (after destaining) by nine different methods (Ziehl-Neelsen, Andrejew, Herman, Konrich, Gasis, Fontes, Burke and Dunning, Ehrlich, and Spengler) emphasized further the importance of technic in the staining of tubercle bacilli. Much's (Gram) method alone proved unsatisfactory with sputum on account of the difficulty of differentiating tubercle bacilli from other small cocci and dye precipitates, since in the majority of cases only the granules stain. Double bacillary staining (carbolfuchsin—Gram Much) of sputum and pus did not increase the efficiency of the simple single bacillary (carbolfuchsin) stain but increases the cumbersomeness of the technic. For practical purposes the simpler methods have the advantages of speed and simplicity. The finding of acid-fast bacilli (with the usual clinical precautions) practically certifies a diagnosis of tuberculosis by the simple methods, while the presence of Gram (Much) granules alone, requires corroborative positive animal inoculation findings to certify the diagnosis. It is for this reason that the Gram (Much) granules and combined methods are mainly of academic interest. It does not seem that the Gram granules differ from the granules seen in appropriately stained carbolfuchsin preparations since their numbers, morphology and location appear to be identical. Preparations stained first by the Gram (Much) method followed by the modified Ziehl-Neelsen carbolfuchsin-acid alcohol technic reveal only blue granules in a red bacillus. Red and blue granules in a lighter red bacillus were never noted. For practical purposes the simple steaming carbolfuchsin (Ziehl-Neelsen) method for staining tubercle bacilli, or one of its modifications, is recommended to the clinical pathologist. The choice of colors remains with the individual operator since any number of dye combinations can be used for staining and counterstaining tubercle bacilli. A negative finding by the simple method requires for further elaboration animal (guinea pig) inoculation, because as many as a million bacilli (about 0.000.1 mg.) per cubic centimeter material is hardly discernible microscopically while from 10 to 100 bacilli from a culture freshly isolated from sputum suffice to infect a guinea pig. In view of the danger of technical errors and in order to raise the present efficiency of the technic, especially with otherwise negative findings or when only single specimens are available, it is recommended that each specimen tested for tubercle bacilli by staining methods be controlled by

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the coincident staining of a like preparation to which a standard suspension of tubercle bacilli, which can easily be kept available in the laboratory at all times, has been added.

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THE REACTIONS OF TYPHOID VACCINATION*

By Henry J. Nichols, M.D., and A. Parker Hitchens, M.D., Majors, Medical Corps, U. S. Army

Clinical pathologists, in addition to other qualifications, are expected to be practical immunologists. At least, they are called upon to make and give various prophylactic and therapeutic vaccines and to make skin tests for susceptibility to various foreign substances and parasites. In many instances, it is easier to go through the motions than to explain the chemical and physical mechanism which determines the result. The practical results for the individual and for civilization also often speak for themselves, even in the absence of a complete analysis. But we lose our professional and scientific attitude and become mere technicians unless we try, no matter how unsuccessfully, to understand what we are doing.

The immunologic procedures for which we have the most convincing explanations are those concerned with antitoxic immunity as seen in the Schick and Dick tests and in the corresponding measures for active and passive immunization. Even here, however, the question of bacterial immunity is either ignored or disregarded, as the toxin-antitoxin factor is a decisive one in diphtheria and largely so in scarlet fever. The factor of bacterial immunity is still present, however, as is illustrated by healthy carriers of pathogenic diphtheria bacilli who have a positive Schick test. In other words, they have no antitoxic immunity but apparently plenty of antibacterial immunity. This factor is also illustrated by the septic complications of scarlet fever patients who have been cured of the toxic symptoms by active or passive antitoxic immunity. In the Schick and Dick tests we also have pseudo-reactions which increase with age and which have not been correlated with any immune processes. The early reactions in vaccination against smallpox are believed to be of significance in detecting immune individuals and to be parasitic in nature, but here we are dealing with a distinctive factor, namely, the living organism.

The recent advances in our knowledge of the problems of antitoxic immunity only emphasize our ignorance on questions of bacterial immunity, as is seen in typhoid vaccination. This procedure has prevented much sickness and death and made the world a safer and better place to live in, but many theoretical and practical questions remain.

At the Army Medical School during the last fifteen years, we have made a very large amount of typhoid vaccine, and naturally have seen and heard a good deal about reactions. We have heard about reactions following

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broken-off needle points, of reactions following intramuscular, intravenous and intracutaneous injections, and of reactions following doses varying from 1.0 c.c. upward. Of course, these are incidentals. The ordinary reactions in the average healthy person, even if not understood chemically, are well enough known clinically so that little need be said of them. Among the millions of persons injected with the vaccine from the School, a few unusual reactions have been reported. Even a few fatalities have been coincidental with, or more or less directly connected with, an injection of vaccine. Hence, we have probably had an unusual opportunity of trying to answer questions. In presenting some considerations on this subject, it is not with the hope of directly answering the many questions raised but with a view to restating problems which further work will eventually solve.

In the first place, can we tell who needs to be vaccinated or revaccinated? Undoubtedly, some persons are immune as a result of a previous attack of the disease or a previous vaccination, or possibly by reason of hereditary make-up, or on account of age. Various tests for specific immunity have been proposed, but these tests are not accepted today as sufficiently reliable for the selection of susceptibles. The skin tests are complicated by nonspecific factors and no great confidence can be placed in them. Even if purely specific we cannot interpret them in terms of real phagocytic or humoral immunity. An individual may have an hereditary horse asthma without being a good horseman, and in the same way a person may give reaction to substances in the typhoid bacillus without being able to master the bacillus in his tissues. There are antibodies in the serum, such as agglutinins, precipitins, and complement-fixing bodies, which we can measure, but we cannot say that their presence indicates protection, either clinically or experimentally.

If nonspecific and little understood specific factors complicate the question of bacterial immunity, the question of antitoxic immunity is just as difficult. No true toxins in appreciable quantities have been isolated and no potent antitoxin is available. To say "endotoxin" is to admit the whole difficulty. Gross variations in response to the toxic elements are easily demonstrated on different individuals, but the significance of these reactions in terms of immunity is not yet realized. The only answer that can be given to the questions, therefore, is one based on the clinical observation. A previous attack of the disease usually confers a high immunity for life and such persons need not be vaccinated. Of course, the actual nature of the previous infection should be authenticated, if possible. Well-vaccinated individuals are also protected for an indefinite time. In the army we now give two courses of three injections each with an interval of three years. At present, only clinical observation can tell us the limits of this acquired immunity. Some reliable test of immunity is much needed.

The second question which often arises is this: Does a sharp reaction indicate that the individual needs the vaccination, or the opposite? If we used the analogy of the Schick and Dick tests, a marked local and general reaction would be interpreted as an evidence of lack of immunity and the need of vaccination. But if we look at the reaction as antibacterial or aller-

gic, we would conclude that the patient is highly protected and does not need the vaccination. If we admit a third possibility, as we must, namely, one of nonspecific reaction, we reach the conclusion that these three factors are so intermingled that a satisfactory answer to this question cannot as yet be given.

There is a related question, namely: Is it possible to protect with a nontoxic vaccine? As far as can be told at present, the answer is in the negative. The so-called endotoxin seems in some way necessary for the immunization and has not been separated from the bacterial proteins. It is difficult to believe that a nontoxic vaccine can produce an immunity comparable with that which is acquired during the disease, only at the expense of weeks of severe sickness. We have worked with various fractions in order to try to separate the bacterial and toxic factors, but our purest bacterial fractions seem to have a toxic element, and our purest toxic fractions seem to have a bacterial element. Apparently these questions will remain unanswered until the physiologic chemist or physicist with an x-ray-like technic can see through the complexities of the structure of the bacillus and separate the fractions in usable forms

Leaving these more fundamental questions, Major A. P. Hitchens and I have recently asked ourselves the more modest and practical question: Is it possible to foretell a severe reaction? In general, severe reactions are of two kinds, one which comes in a few hours, and the other which reaches its maximum effect in a few days. An example of the first is as follows:

"A man received his first injection November 28, 1923, without marked reaction. He had previously received two injections, one in 1915 and one in 1918. The reactions were always severe but not as bad as the present one. On December 5 he received a second dose at 10 A.M. One hour later, he had a chill. Two hours later he was very ill, cyanotic, pulse 100, weak and rapid, respiration 30, skin cold, and having a hard chill. He was put to bed and given 1.0 c.c. of adrenalin hypodermically. At 12 o'clock he vomited about two ounces of fresh blood. He vomited again at 12:30, and at 1 o'clock about one ounce of blood. Patient's color began to improve shortly after administration of adrenalin, and all alarming symptoms disappeared. At 2 o'clock his temperature was 104; from then on the patient recovered. The physicians who took care of the case thought it was doubtful if he would have recovered without prompt treatment."

This case, of course, cannot be considered as one of frank anaphylaxis, but we have had two other cases of similar nature, following the second dose, in which an allergic element must apparently be considered. Such cases should, of course, be differentiated from those in which the vaccine happens to enter a vein. The symptoms may be somewhat the same. True anaphylaxis has been reported with typhoid extracts in the guinea pig, but we have never been able to reproduce it with whole vaccine. We have not yet had or made an opportunity to try the passive transfer of anaphylaxis from a susceptible person to a nonsusceptible or to an animal. These reactions can at least be called anaphylactic-like, or anaphylactoid. They are extremely rare but the practical point is that adrenalin should be a part of the equipment for vaccination.

The second type is more toxic in nature, sometimes with symptoms of enteritis, which come on in twenty-four to forty-eight hours.

It may be said that in these reactions the vaccine is not at fault per se. Usually 20 to 200 men are vaccinated at the same time, from the same lot of vaccine, and only the occasional susceptible individual is picked out. As in the administration of arsphenamine or other drugs and serums, the difference in effect depends on the individual. This is reassuring to the vaccine maker, but does not help the vaccinator.

In pursuing the plan of attempting to pick out the bad reactions, we made intracutaneous injections in the arm of ordinary vaccine diluted at least to 100,000 organisms for the skin dose, and read the immediate reactions as in sensitization tests and the late reactions after twenty-four to forty-eight hours as possible indicators of the two types of reactions. We then attempted to correlate these reactions with various factors.

The results are given in Table I:

TABLE I

IMMEDIATE AND LATE SKIN REACTIONS IN RELATION TO REACTIONS FOLLOWING TYPHOID VACCINATION

(Suspension of Vaccine, 100,000 Bacilli in 0.1 c.c.)

	NO.	IMMEDIATE REACTION	24-HOUR REACTION
Previous typhoid fever	8	2.2	1.7
No typhoid or vaccination	21	1.	1.9
Previous vaccination	59	1.2	1.2
Other protein sensitizations	6	1.5	1.5
Before vaccination	28	1.2	0.8
3 months after	28	1.8	1.8

Figures indicate average size of reaction based on four plus system of reading.

The immediate skin reaction was marked in those who had had typhoid fever. Recent vaccination also increases the immediate reaction. Other sensitiveness also tends to increase the immediate reaction. These results give some indication of allergy, but they are not marked or entirely consistent. The late or toxic reactions are also suggestive, as those persons with no history of typhoid or vaccination give low immediate skin reactions and high late reactions. This conclusion does not hold true generally, however, as those recently vaccinated also give good late reactions. The most that can be said is that the results are suggestive of two factors, one bacterial and the other toxic.

The most definite correlation was between the immediate skin reaction and the general vaccination reaction, which ran fairly parallel. No connection could be seen between the late skin reaction and the vaccination reaction:

	IMMEDIATE SKIN REACTIONS	VACCINATION REACTION
(–) or single plus	13	31%
double plus or triple plus	21	65%

In other words, the allergic factor seems to be the most important in reactions. This conclusion fits in with clinical experience, which tells us that severe reactions are more apt to occur in those who have had typhoid fever or have been repeatedly vaccinated. It must be noted, however, that nonspecific reactions increase in severity with age, and the subject remains complex.

It has been thought that the paratyphoid fractions are more toxic than the typhoid fraction, and the Navy at present uses a monovalent typhoid vaccine of 1,000 million per c.c. We have tried the three fractions by skin reactions and by vaccination in separate arms and have been unable to find any essential differences.

In view of the apparently toxic and antitoxic element in some individuals, we have attempted to neutralize the toxic factor by mixing vaccine and serum from apparently antitoxic individuals and trying skin tests on susceptibles. Six tests with proper controls were made in which four showed a definite neutralization of toxin with serum from a nonreactor. The nature of this neutralization is difficult to explain as antiendotoxins are not generally accepted. The subject is complicated by sensitiveness of the skin of some individuals to the injection of serum or even of salt solution.

One definite conclusion is the obvious one that the strength of the vaccine materially affects both early and late reactions. Soon after the war, we revised our plant and technic and put the vaccine on the theoretical strength of 2,500 m. per c.c., assuming that the Counting Chamber technic gave the same results as the Wright method. We immediately found that the number of reactions increased. We have since found that the Counting Chamber technic gives a stronger vaccine, because the count is lower and the dilution less. In the Wright technic many red cells are lost and the dilution is greater than it should be. We have now reduced our vaccine to 1,000 m. per c.c.; 500 m. typhoid, 250 m. *A*, and 250 m. *B*, which is equivalent to a count of about 2,000 by the Wright technic, and we believe more nearly accurate. Practically all complaints have ceased since this reduction.

In general, it can be said that there are at least three factors in the reactions: one specific and bacterial, a second specific and toxic, and a third which is nonspecific. While one or the other element may be shown to predominate in the picture, they cannot be sharply separated. The practical conclusion is that the clinical pathologist has the arduous occupation of being both a clinician and a pathologist. We must individualize our subjects for vaccination and be prepared for various reactions. In case of doubt, the usual procedure is to cut the dose and increase the number of injections. We have analogy in support of this procedure but no actual figures on protection. A skin test may help in detecting severe reactors.

In clearing up some of these questions, the following lines of work seem to be the most promising:

1. The separation of distinct fractions of the bacillus by competent chemists.

2. The skin test of these fractions with proper controls on typhoid fever patients and convalescents and in normals of different ages and on vaccinated individuals.

3. Attempts to transfer and neutralize skin reactions by using serum from reactors and nonreactors.

4. A more critical clinical study of ordinary reactions.

SUMMARY

1. Recent advances in dealing with antitoxic immunity as seen in diphtheria and scarlet fever have not been duplicated in regard to bacterial immunity as seen in typhoid fever.

(a) It is not possible at present to detect susceptibles and immunes to typhoid fever by skin or other tests.

(b) The significance of reactions to vaccination in terms of immunity is not yet clear.

(c) The question of toxic and nontoxic vaccines is not yet satisfactorily answered.

2. Severe vaccination reactions are of two kinds, immediate or anaphylactic-like, and late or toxic.

3. Immediate and late skin reactions with vaccine give some suggestions of two factors in reactions, (a) bacterial or allergic, (b) toxic.

4. There is also a marked nonspecific element.

5. These factors are so mixed together that an analysis of reactions in terms of immunity is not possible at present.

6. Severe reactors may in some instances be picked out by immediate skin reactions.

DISCUSSION

Dr. Frank M. Huntoon.—Every now and then some one comes along with common sense, and it rather startles us. There are many things upon which one could disagree with Dr. Nichols, on his theoretical ground, and on his question whether the toxic element is necessary for immunization. Neither of us has any proof. I would not give up hope of producing such a vaccine. It seems to me that the old problem of the nature of the immunity produced here by the natural disease or by immunization lies in the question that Dr. Kolmer brought up: Is it a strictly humoral immunity? This again leads to the question of the method of infection in typhoid fever. Is typhoid primarily a disease of the intestines or does it get to the intestines through the blood stream? Now there is another question which may be brought up: Is it necessary to have antibody in the blood in order that the patient may be immune from the actual typhoid? I do not think so. It is a fact that you can inject an animal with typhoid serum and measure the time it takes for antibodies to appear in the blood. If you allow that animal to remain untreated until the antibodies have disappeared, and then if you reinject it and test it every day to see when the antibodies reappear, you can probably find them in twenty-four hours. The paper by Dr. Nichols points the way to some very useful and needed work, work that does not have to be done in highly organized, complete laboratories, but anywhere.

Dr. A. S. Brumbaugh.—In 1918 at the Army Medical School, one of the medical officers originated a typhoid vaccine made up in oil that was supposed to have the advantage that the whole immunization could be administered at one time and which was not supposed to cause any reaction. I would like for Dr. Nichols to tell us what has been done about that.

Dr. Robert A. Kilduffe.—I do not wish to discuss this paper; as usual, I am in search of information.

Dr. Nichols has called our attention to the fact that it is a very difficult thing to measure in any way the degree of immunity to typhoid fever, and it is well known that the agglutinin-titer is not a measure of immunity.

Sometime ago Dr. O'Hara, of Boston, described the study of a small series of typhoid fever cases by frequent quantitative agglutination tests and suggested, as a result of his

observations, that when the agglutinin-titer began to drop in the face of unchanged or unfavorable clinical findings, that the dropping titer was a possible indication of an unfavorable prognosis.

I was fortunate enough to be able to try this out and, though my series was very limited, this suggestion was apparently borne out: the cases in which the titer showed a sudden or persistent drop, died.

I have never seen any further report or note upon this phase of the agglutination test in typhoid fever. Knowing the immense field covered by the records of the army in this branch of work, I would like to ask Dr. Nichols if any work has been done in the army along this line or if they have any information as to the availability of this procedure as a possible prognostic criterion.

Dr. Robert A. Keilty.—We are continuing to give typhoid vaccine without thinking of fatality. There were possibly a million doses given, with one fatality, and I do not know whether it is in the records or not. In four autopsies there was nothing at all to account for death.

Dr. John A. Kolmer.—One subject of considerable practical importance in relation to Dr. Nichols' paper is the matter of developing a satisfactory test for natural or acquired immunity in typhoid fever. I agree with Dr. Nichols that the skin test has not fulfilled expectations and hopes in this direction; I also doubt the value of the agglutinin reaction for this purpose, but, inasmuch as a goodly part of immunity to the typhoid and paratyphoid bacilli would appear to be humoral in nature, I am hopeful that investigations will ultimately evolve a bactericidal test in vitro applicable to the whole blood as a satisfactory measure of immunity to serve as a practical guide to vaccination and revaccination in this disease.

Dr. H. J. Nichols (closing).—Dr. Kolmer has spoken of the typhoidin test; it is certainly to be hoped and expected that we will soon have some kind of skin test which will indicate the necessity or lack of it for vaccination. Experimentally, protection tests are the most convincing. Determination of antibodies is not so conclusive. The guinea pig has a natural infection with a Para *B*-like organism, B. aertrycke, and it is possible to carry out actual protective experiments under different conditions with this organism. We have done some of this work and reported it in the Jour. Exp. Med., 1923, xxxviii, 283. Lipovaccine has been referred to. This was given up on account of difficulty of making a sterile product and a poor showing experimentally.

We have had several fatalities, such as have been mentioned; in one we found a very high degree of calcification of the coronary artery. One occurred among a number of men who had food poisoning at the time of vaccination. All our evidence in these cases points to some special weakness in the individual rather than defect in the vaccine and argues for individualizing our prophylactic as well as our therapeutic vaccines.

I feel that we are actually just starting upon a real understanding of this general subject. As Dr. Huntoon says, it does not require any elaborate technic or equipment to progress. We will get somewhere if we simply observe with open eyes. Of course, all that is learned in the finer analysis of reactions like the tuberculin test and other skin tests will help to give the final answer.

BLOOD COUNTS IN MISSISSIPPI*

By Leon S. Lippincott, M.D., Vicksburg, Miss.

IN THE last few years several observers have called attention to the fact that there is no standard normal total leucocyte count or differential leucocyte count that holds good for all localities. It was in the belief that an investigation of differences should be carried further and with the idea of establishing standards for our work in Mississippi that the study covered by this paper was attempted.

Most textbooks give the total leucocyte range for normal individuals as from 5,000 to 10,000. The normal differential count is given as varying as follows:

Small mononuclears	20 to 25%
Large mononuclears (including transitionals)	2 to 11%
Polymorph. neutrophiles	60 to 75%
Polymorph. eosinophiles	1 to 5%
Polymorph. basophiles	0.5%

The counts here considered, 3,045 in number, were made on hospital and clinic patients, many of them as a part of the routine laboratory examinations. The records were taken as they came, with no attempt to pick cases. The differential counts were made from Wright's stain preparations. Counts with eosinophile percentages above five were not included as such counts in our experience generally indicate animal parasites. Counts from white and colored patients were listed separately to determine any possible differences due to race. Children's counts were not included.

Total leucocyte counts within the so-called normal range of 5,000 to 10,000 were first considered, with the following average findings:

Averages of 1500 Counts on White Patients Showing Leucocytes 5,000 to 10,000

Total leucocyte count		7,638
Small mononuclears	30.60%	
Large mononuclears	6.50%	
Polymorph. neutrophiles	61.85%	
Polymorph. eosinophiles	0.95%	
Polymorph. basophiles	0.10%	

Averages of 300 Counts on Colored Patients Showing Leucocytes 5,000 to 10,000

Total leucocyte count		7,395
Small mononuclears	33.55%	
Large mononuclears	4.95%	
Polymorph. neutrophiles	60.50%	
Polymorph. eosinophiles	0.95%	
Polymorph. basophiles	0.05%	

It will be noted that the small mononuclears are considerably above the usual maximum normal standard and the polymorphonuclear neutrophiles

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near the usual normal lower limit. The average eosinophile percentage is less than one, although actually we rarely are able to demonstrate intestinal parasites unless the eosinophile percentage is five or more. The basophiles are much lower than the usual standard normal.

As the above counts are from hospital and clinic patients, it is possible that there may have been inflammatory conditions present in some instances, which would raise the neutrophile percentage; and malaria or typhoid in others, which would lower it. It is felt that these two conditions would possibly offset each other.

From observation of clinical conditions and findings I am convinced that in a great majority of cases a total leucocyte count above 8,000 in Mississippi means a leucocytosis and a neutrophile percentage above 65 means an increase over normal. In making this statement, I do not ignore the fact that there are normal variations.

There appears to be no marked difference between the counts of white and colored patients. The mononuclears are slightly lower in whites and the polymorphonuclear neutrophiles slightly higher. Basophiles were scarce in colored patients.

At least one observer has noted that persons who have lived six weeks or longer in high altitudes show a low leucocyte count with increased mononuclears and low polymorphonuclear neutrophiles. Altitude could not be considered as a factor in the counts recorded here as Mississippi is generally low.

TOTAL LEUCOCYTE COUNTS BELOW 5,000

When counts below 5,000 are considered, the average total leucocyte counts and differential counts are found to be as follows:

AVERAGE OF 100 COUNTS ON WHITE PATIENTS SHOWING LEUCOCYTES BELOW 5,000

Total leucocyte count		4,182
Small mononuclears	35.1%	
Large mononuclears	11.5%	
Polymorph. neutrophiles	52.5%	
Polymorph. eosinophiles	0.8%	
Polymorph. basophiles	0.1%	

AVERAGE OF 5 COUNTS ON COLORED PATIENTS SHOWING LEUCOCYTES BELOW 5,000

Total leucocyte count		4,450
Small mononuclears	34.0%	
Large mononuclears	6.2%	
Polymorph. neutrophiles	58.4%	
Polymorph. eosinophiles	1.4%	
Polymorph. basophiles	0.0%	

In these low counts the mononuclears are increased at the expense of the neutrophiles. Basophiles were again low.

TOTAL LEUCOCYTE COUNTS ABOVE 10,000

Counts above 10,000 are considered in three groups: those from 10,000 to 15,000; those from 15,000 to 20,000; and those above 20,000, the counts of white and colored patients being given separately, as follows:

CHEMICAL INVESTIGATIONS ON NEOARSPHENAMINE*

I. Methods of Analysis

By Louis Freedman, Ph.D., Brooklyn, N. Y.

Although neoarsphenamine has now been in extensive clinical use for over ten years and its therapeutic importance has long been established, the published work on its analysis and chemical composition is both limited and inconclusive. Neoarsphenamine was first described in the patent literature[1] as a condensation product of salvarsan with sodium formaldehyde sulphoxylate, while the circulars accompanying the original preparation, as manufactured by the Farbwerke Hoechst in Germany, described it as the monomethylene sulphinate of salvarsan. The lowered arsenic content of 19 per cent from the theoretical of 32 per cent was accounted for by the presence of inert salts. Commercial preparations of neoarsphenamine are generally described as "a compound formed from arsphenamine by the action of formaldehyde sulphoxylate." The above description has been generally adopted by all manufacturers of the drug, and today any product which passes the required toxicity tests and conforms to an arsenic content of at least 19 per cent, regardless of modes or conditions of manufacture, is accepted as neoarsphenamine.

Bertheim in 1913[2] called attention to the possibility of the combination of one or two mols of formaldehyde sulphoxylate with salvarsan resulting in either a mono- or disubstituted product, depending upon the conditions of manufacture.

In 1921, Raiziss and Falkov[3] described a method for the examination of neoarsphenamine from a chemical standpoint and concluded that neoarsphenamine contained both the mono- and disubstituted derivatives. In 1921 and 1922, Macallum[4, 5] published reports on the chemical analysis of domestic and foreign neoarsphenamines and found that there was considerable difference between the American and French products, and that the latter were made up almost entirely of disubstituted derivatives. Voegtlin and Miller[6] in 1922 showed by trypanocidal and toxicologic tests that very wide variations existed for different preparations of neoarsphenamine and emphasized the lack of uniformity in the products. In 1922, Dale and White,[7] in their report to the British Medical Research Council on the biological efficacy of various preparations of the salvarsans, stated that preparations of the neosalvarsan or "914" type fell into two distinct classes. In the first class were those which resembled the German product more or less closely in certain physical characteristics and the rapidity with which they underwent decomposition on exposure to air. The second group consisted of those products which differed from the German product in being m[illegible]y soluble and showing evidence of greater stability [illegible]tion. The [illegible] of the second group were described by Dale and

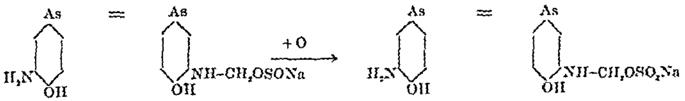
*[illegible]rom the H. A[illegible] [illegible]ies, Inc., Brooklyn, N. Y.
[illegible]ceived for pub[illegible] [illegible] 27, 1925.

White as being of lower toxicity but also inferior in therapeutic activity as compared with the original product of German manufacture.

It would seem, therefore, that these wide variations in the composition of various brands of neoarsphenamine were in part due to the fact that the manufacturers supplemented the specifications of the original method of manufacture, primarily to obtain products of low toxicity, but without much regard to the identity of the resulting products, provided they conformed to the existent requirements. A comparison of the method of preparation described in the original patent specifications (l.c.) with those of Krumweide[8] and Oechslin[9] show how widely they may differ.

It is quite obvious, therefore, that the name neoarsphenamine is used to cover a number of products which are prepared from the same chemicals, but which may differ radically in some of their physical properties and in their chemical composition. These differences are also reflected in wide variations in toxicity and trypanocidal efficiency. It is therefore of great importance for a method of analysis to be developed which will serve to distinguish the different types of neoarsphenamines.

Those authors who published analytical data on neoarsphenamine with the purpose of determining the chemical constitution of the drug, include A. D. Macallum (l.c.), Raiziss and Falkov (l.c.), de Mytennaere[10] and Elvove.[11, 12] Macallum, in his first paper, "Examination of Neoarsphenamines" (l.c.), devised a method for the determination of the relative amounts of the various constituents by a portionwise oxidation of the drug by means of iodine. He found that in the presence of strong mineral acids neoarsphenamine required 12 mols of iodine for complete oxidation, indicating that the formaldehyde sulphoxylate side chain was broken off and completely oxidized. In his second paper, "The Constitution of the French Drugs," Macallum extended his method of analysis by carrying out the procedure successively in acid and alkaline solutions. He claimed by this method to be able to differentiate between the combined formaldehyde sulphoxylate and bisulphite groups and also to determine the varying amounts of these uncombined formaldehyde sulphur compounds. Macallum also stated that the first step in the oxidation of neoarsphenamine was the oxidation of the N-formaldehyde sulphoxylate group to the N-formaldehyde bisulphite group according to the equation,



and he included the two compounds represented by the above formulae, under the name of neoarsphenamine.

Raiziss and Falkov's (l.c.) method for the calculation of the various constituents of commercial neoarsphenamines was based on a partition of sulphur in the drug, and is to a certain extent similar to Macallum's method. The total sulphur was first determined by both the Carius method and oxidation by digesting with iodine for six hours. The difference between the two found

values was accounted for as nuclear sulphur, although no evidence was given that nuclear sulphur was not oxidized by digestion with iodine. The percentages of nuclear sulphur were found to be so small that they may actually have come within the limits of experimental error.

The sulphur of the uncombined formaldehyde sulphoxylate, together with any inorganic sulphates, was determined by oxidizing the filtrate obtained from the precipitation of the free neosulphinic acid by means of hydrochloric acid. The sulphur of the combined sulphoxylate was represented as the difference between the total sulphur (exclusive of nuclear sulphur) and the sulphur of the filtrate. Arsenic was determined gravimetrically. From the results of their analyses, Raiziss and Falkov concluded that neoarsphenamines were made up of mixtures of mono- and disubstituted derivatives and tabulated the various percentages of these constituents, together with the amounts of combined and uncombined formaldehyde sulphoxylates, although they failed to state how they arrived at their conclusions. The authors also failed to observe, as de Mytennaere (l.c.) pointed out in a polemic on their paper, that a certain percentage of the combined sulphur may have been in the form of the oxidized sulpharsphenamine.

In the results recorded by Raiziss and Falkov the sum of the sulphur contents of the various constituents fails to check with the total sulphur found, and many of the calculations do not check arithmetically. For example, in Table V, page 217, for sample D1, the total combined sulphoxylate was found to be 18.6 mg., but when calculated from the figures given for mono- and disubstituted neoarsphenamines, it is equivalent to only 15.9 mg. The total organic arsenical component of D1 was given as 63.8 mg., but from the arsenic content of the sample, which is equivalent to 48 mg. arsphenamine, plus the 18.6 mg. combined sulphoxylate found by analysis, the figure should total 66.6 mg.

Raiziss and Falkov have also stated that the formaldehyde sulphoxylate attached to the amino group is not oxidized by iodine in the cold. This is contrary to the statement of Macallum (l.c.) and also contrary to our own experimental findings, which will be given in detail in the experimental part of this paper.

De Mytennaere (l.c.) studied the chemistry of various neoarsphenamines from the standpoint of the effect of the variation in chemical constitution on the toxicity of the drug. He determined the percentages of carbon, hydrogen, nitrogen, arsenic and sulphur in various commercial samples and also attempted to precipitate the various constituents by fractional precipitation in acetic acid solution, but his results were not very conclusive. He stated that the free neosulphinic acid could not be completely precipitated from compounds containing 10 per cent or more of sulphur; and that he found very little difference between neoarsphenamine and sulpharsenol (sulpharsphenamine).

Elvove (l.c.) described a method for the estimation of sulphur as sulphate in neoarsphenamine, for the purpose of determining, together with other analytical data, the distribution of sulphur in the drug. He (l.c.) also described a method for the determination of the total sulphur in neoarsphenamine and sulpharsphenamine which depends on the oxidation of the sample

by means of potassium permanganate and hydrochloric acid. This latter method is said to have the advantage over the Carius or peroxide-fusion method because of its simplicity of operation and because the procedure is similar to that used for the determination of arsenic, so that the latter may be determined in the same sample of the drug.*

The above articles include practically all the available data regarding the investigations of neoarsphenamine from a chemical standpoint. In the experimental part which follows, we have, by attempting to apply these methods, been able to show that certain of them are based on certain fallacies and consequently cannot give accurate results.

EXPERIMENTAL PART

In the study of a series of different commercial preparations of neoarsphenamines which were under investigation in this laboratory, we attempted to utilize the methods described by both Macallum, and Raiziss and Falkov, by combining the essential operations of both methods without changing them in principle. In this way the analyses were carried out with a minimum amount of manipulation and the use of a minimum amount of material.

Arsenic was determined according to Lehmann's method (l.c.), and the total sulphur by Elvove's (l.c.) method. The total oxygen requirement, which is the same as the total reducing power in Macallum's method, was obtained by titration of 0.2 gram of the sample dissolved in 30 c.c. air-free water, with 0.1 N iodine solution. One c.c. 0.1 N I is equivalent to 0.8 mg. oxygen. All precautions to avoid possibility of oxidation by the air were taken. The oxygen requirements of the filtrates, i.e., of the uncombined sodium formaldehyde sulphoxylate, were obtained, precisely as described by Macallum (l.c.) and Raiziss and Falkov (l.c.), by precipitating the free neosulphinic acid with dilute hydrochloric acid and titrating the filtrates with the standard iodine solution. The analyses for the sulphur partition of the samples, according to the method of Raiziss and Falkov were also made on portions of these same filtrates. The results obtained by the above combined methods of analyses, which are given in Table I, did not give satisfactory and concordant results as claimed by the authors of these methods. The inconsistencies in the above results, together with many striking observations made during the course of the analyses, prove the impracticability of the methods. We found that the amount of iodine required for complete oxidation of neoarsphenamines increased as the acidity of the solution increased; that different amounts of acid were required to precipitate completely the different free sulphinic acids from their respective sodium salts;† and that at least one brand of neoarsphenamine could not be completely precipitated except by the use of concentrated hydrochloric acid and allowing the solution to stand until the filtrates no longer became turbid. We have also found that the determination of sulphur in the filtrates has no quantitative value, because the neosulphinic acid is more or less unstable in acids strong enough to make a precipitation anywhere near com-

*A third paper by Elvove[11], "A Method for the Examination of Neoarsphenamine and Sulpharsphenamine," appeared after this article was written. This paper will be discussed by us in a subsequent publication.

†The free acid precipitated from aqueous solutions of neoarsphenamine will be referred to hereafter as neosulphinic acid.

plete, the formaldehyde group being split off the N-organic nucleus with the formation of arsphenamine hydrochloride.

As the methods of Macallum and Raiziss and Falkov are based directly on the assumptions that arseno groups can be titrated quantitatively by iodine in an acid medium and that the free sulphinic acid of neoarsphenamine is quantitatively precipitated by means of hydrochloric acid without decomposition, it is evident that these methods cannot give quantitative results.

Since some of the figures given in Table I are obviously incorrect, we did not use them as a basis for calculating the various constituents of the sample as detailed in the method of Raiziss and Falkov (l.c.). In this table the difference between the total oxygen requirements (Column 5) and that required by the trivalent arsenic (Column 6) is the oxygen required by the uncombined formaldehyde sulphoxylate (Column 7). This should check with the oxygen requirements of the uncombined formaldehyde sulphoxylate as calculated by titration of the filtrates with iodine (Column 9). In all of the samples, with the exception of Sample F, the oxygen obtained by calculation (Column 7) is greater than that obtained by actual titration, this difference (Column 10) varying considerably in each case.

Assuming that commercial neoarsphenamines, besides the actual arseno compound and sodium formaldehyde sulphoxylate, contain nothing which will be oxidized by iodine, the combined formaldehyde sulphoxylate groups attached to the amino groups therefore must react with iodine, being oxidized to the formaldehyde bisulphite groups. Thus a disubstituted neoarsphenamine should require more iodine than a monosubstituted product. This difference in oxygen requirements (Column 10), however, does not check with the calculated combined formaldehyde sulphoxylate for each sample of neoarsphenamine, even though it is assumed that only monosubstituted neoarsphenamine is present. It is therefore evident that some other factor or factors are involved in this method of analysis which tends to give such discordant results.

EFFECT OF ACIDITY ON TITRATIONS OF ARSENO GROUPS WITH IODINE

The variability of the results obtained by the previously described methods led us to investigate the effect of acidity on the titrations of the arsenicals with iodine, both with regard to the arseno groups alone and when combined with side chain groups, as in neoarsphenamine.

It has been definitely shown that the trivalent arsenic in arsphenamine is practically quantitatively oxidized to the pentavalent arsenic acid by means of iodine in neutral or very slightly acid solutions.[15, 16] In the presence of an excess amount of mineral acid, however, we have found that the oxygen requirement of the arseno group decreases, less iodine being required for complete oxidation to pentavalent arsenic, and that this decrease is proportional to the increase in acidity of the titrated solution.

Table II shows that, when the titration with iodine is carried out in a solution, the final acidity of which is approximately 1.0 per cent, only 97 per cent of the theoretic iodine is required. When the acidity is further increased to 3.24 per cent, 91 per cent of the theoretic iodine is required to completely

TABLE I

ANALYTICAL DATA OF COMMERCIAL NEOARSPHENAMINES OBTAINED BY COMBINATION OF METHODS OF MACALLUM AND RAIZISS AND FALKOV

SAMPLE OF NEOARSPHENAMINE*	% AS IN ORIG. SAMPLE	% S IN ORIG. SAMPLE	C.C. N/10 I PER 100 MG. OF SAMPLE	MG. OXYGEN REQUIRED PER 100 MG. NEOARSPHENAMINE			C.C. N/10 I REQ. BY FILTRATE PER 100 MG. NEO.	OX. REQ. BY FILTRATE CALC. EX. I TITRATION	MG. OX. DIFF. BETWEEN CALC. AND FOUND.	SULPHUR DISTRIBUTED IN FILTRATE			
				TOTAL OX. CALC. EX. I REQ.	OX. CALC. EX. AS	DIFFERENCE OX. REQ. BY FILTRATE				TOTAL S CALC. BY DIFF.	TOTAL S FOUND	S FOUND AS Na_2SO_4	S AS FREE Na F. S. OX., Na F. B. S., ETC.
A	19.26	11.57	17.63	14.11	8.21	5.90	5.73	4.58	1.32	7.46	8.46	2.06	6.40
B	20.15	11.35	17.08	14.46	8.60	5.86	5.50	4.40	1.46	7.06	8.18	3.23	4.95
C	20.06	7.23	16.78	13.44	8.78	4.66	4.98	3.98	0.68	2.96	5.04	0.87	4.17
D	18.85	10.55	18.03	14.42	8.04	6.38	5.17	4.14	2.24	6.54	6.85	2.33	4.52
E	19.22	9.04	16.33	13.06	8.20	4.86	4.08	3.27	1.59	4.84	6.61	1.88	4.73
F	19.68	11.07	11.48	9.18	8.39	0.79	1.25	1.00	−0.21	6.87	4.35	0.56	3.79
G	19.65	9.01	16.46	13.16	8.37	4.79	4.69	3.75	1.04	4.82	6.49	1.71	4.78

*These samples are representative of the different commercial brands of neoarsphenamine obtainable in the open market.

Abbreviations: Ox. = Oxygen; Req. = Requirements; Calc. = Calculated; ex. = from; Na F.S.Ox. = Sodium Formaldehyde sulphoxylate; Na F.B.S. = Sodium formaldehyde bisulphite.

TABLE II

EFFECT OF ACIDITY ON TITRATION OF ARSPHENAMINE WITH IODINE

AMOUNT OF SAMPLE IN GM.	AMOUNT OF HCl ADDED	FINAL ACIDITY OF SOLUTION AFTER ADDITION OF IODINE	N/10 I* REQUIRED PER 100 MG. ARSPHENAMINE	OXYGEN EQUIVALENT PER 100 MG.	DECREASE IN MG. OXYGEN REQUIRED	% DECREASE
0.2	0	0.5%†	16.35	13.08	–	–
0.2	0	0.5†	16.33	13.06	–	–
0.15	0	0.5†	16.40	13.12	–	–
0.2	23 c.c. 1.8%	0.87	16.16	12.92	0.21	1.5
0.2	30 " "	1.0	16.08	12.86	0.28	3.0
0.2	30 " "	1.0	16.00	12.80	0.34	3.0
0.1	10 " 18. %	3.24	15.00	12.00	1.14	9.0
0.1	20 " "	6.24	11.00	8.80	4.34	33.0
	ACETIC ACID					
0.1	20 c.c. 30%	10.0%	16.20	12.96	0.18	1.3
0.1	20 c.c. 100%	33.0	15.95	12.76	0.38	2.7

NOTE: Total volume of solution including added acid, but before addition of iodine, was 30 c.c. per 0.1 gm. arsphenamine. Excess iodine titrated back after 3 minutes, with standard thiosulphate solution using starch as indicator.

*The theoretical number of c.c. of N/10 I required for this sample containing 30.82 per cent As is 16.45, which is equivalent to 13.14 mg. oxygen.

†This acidity which is due to the HI formed by reduction of the iodine, together with that of the HCl of the arsphenamine hydrochloride, (which is so little as to be negligible) may be responsible for the slight decrease in oxygen requirement from the theoretical.

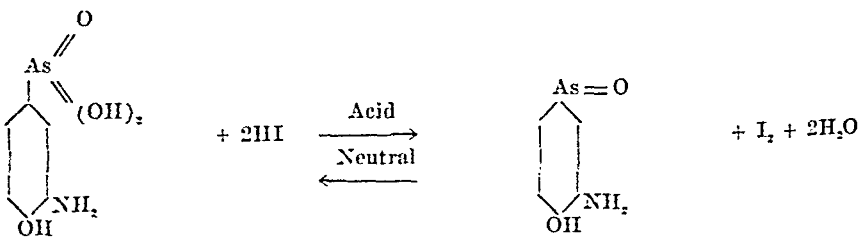
oxidize the arsenic; and in a solution containing approximately 6.0 per cent HCl, only 67 per cent of the theoretic iodine is required.

Acetic acid also has a tendency to decrease the amount of oxygen required by the trivalent arsenic, but to a much smaller degree than hydrochloric acid. Thus in a 33 per cent acetic acid solution, the amount of oxygen required by iodine titration was about 97 per cent of the theoretic, showing that acetic acid has very little effect.

These results indicate that the reaction between the arseno compound and iodine is a reversible one in the presence of mineral acids, similar to the reversible reaction taking place when arsenious acid is titrated with iodine in high hydrion concentration according to the equation:

$$H_3AsO_3 + I_2 + HO_2 \xrightleftharpoons[\text{Acid}]{\text{Neutral}} H_3AsO_4 + 2HI$$ [17, 18]

The corresponding reaction of the arsenic acid may be written thus:



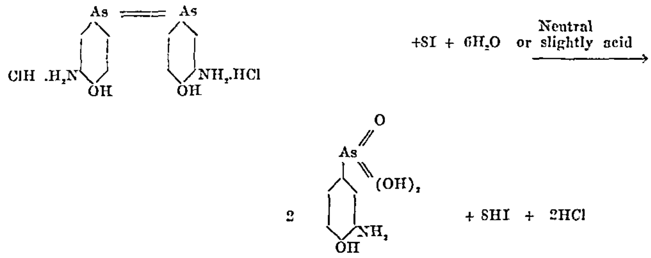
EFFECT OF ACIDITY ON TITRATIONS OF NEOARSPHENAMINE WITH IODINE

As neoarsphenamine is supposed to contain the same arseno grouping as arsphenamine, we would expect to get similar results on titration of solutions of neoarsphenamine with iodine as we get with solutions of arsphenamine.

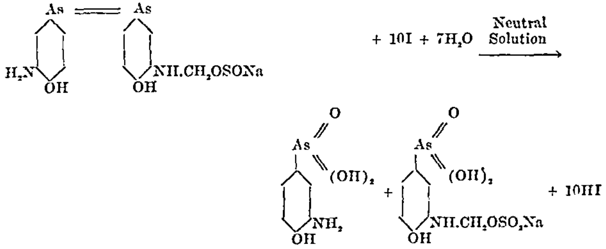
However, actual experimental results show the reverse to be true, i.e., slightly more iodine is required as the concentration of acid is increased. This is due, not to any different reaction taking place with regard to the trivalent arsenic groups, but to the fact that the combined N-formaldehyde-sulphoxylate group is progressively hydrolyzed as the concentration of hydrions increases and the liberated formaldehyde-sulphoxylate group is then completely oxidized by the iodine. The decrease in oxygen requirements caused by the reversible reaction of the arsenic acid with III is more than compensated for by the increase in oxygen required by the liberated formaldehyde-sulphoxylate group.

The formaldehyde-sulphoxylate group attached to the amino group in neoarsphenamine is a strong reducing agent, since it quantitatively reduces methylene blue and is itself oxidized to the corresponding bisulphite group, as will be

(I)



(II)



(III)

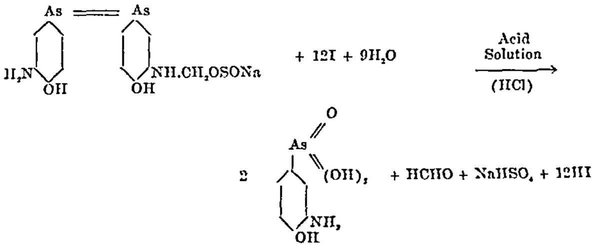


TABLE III

EFFECT OF ACIDITY ON TITRATIONS OF NEOARSPHENAMINES WITH IODINE

(A) HYDROCHLORIC ACID

AMT. OF SAMPLE GM.	AMOUNT OF ACID ADDED	% ACIDITY OF SOLUTION	% FINAL ACIDITY AFTER ADDITION OF I SOLUTION	C.C. N/10 I REQUIRED PER 100 MG. NEO.	MG. OXYGEN EQUIVALENT PER 100 MG. NEO.	MG. OXYGEN TO BE COMPENSATED BY REVERSE REACTION*	TOTAL ADJUSTED OXYGEN REQUIRED	INCREASE IN MG. OXYGEN
0.2	–	–	–	17.40	13.92	–	13.92	–
0.2	1.0 c.c. 18%	0.6	0.24	17.47	13.97	–	13.97	0.05
0.2	2.0 " "	1.12	0.45	17.57	14.05	0.21	14.26	0.34
0.2	5.0 " "	2.57	1.12	17.67	14.13	0.34	14.47	0.55
0.2	7.0 " "	3.40	1.54	17.70	14.16	0.57	14.73	0.81
0.2	10.0 " "	4.50	2.10	17.73	14.19	0.80	14.99	1.07
0.2	15.0 " "	6.0	3.00	17.79	14.22	1.00	15.22	1.30
0.2	20.0 " "	7.2	3.80	17.93	14.34	1.50	15.84	1.92
0.2	20.0 " 30%	12.0	6.32	No end Pt.				

(B) ACETIC ACID

0.2	20.0 c.c. 10%	5.0	2.5	17.45	13.96			
0.2	20.0 " 30%	12.0	6.0	17.32	13.85			
0.2	20.0 " 100%	40.0	20.0	16.80	13.44			
0.2	30.0 " "	50.0	25.0	16.47	13.18			

NOTE: In the above experiments, the 0.2 gram sample was dissolved in 30 c.c. H_2O, and 45 c.c. Standard iodine solution were used in each determination, the excess iodine being titrated back with $Na_2S_2O_3$ solution after 3 minutes.

shown in a subsequent publication. Theoretically there should be no doubt that this same group is oxidized by as strong an oxidizing agent as iodine, even in neutral solution in the cold, and our experimental results prove this to be true. However, as a certain amount of HI is formed during the titration with iodine by the reduction of the iodine, it is impossible theoretically to carry out this titration in absolutely neutral solutions. Although the actual acidity due to the formation of the HI is slight, as shown in Table II, it causes a slight amount of splitting off of formaldehyde-sulphoxylic acid and a resulting increase in the oxygen requirement. The equation representing the above reactions may be written as shown on p. 535.

Acetic acid, however, has no effect on the N-formaldehyde-sulphoxylate linking, and only the arseno group is oxidized by iodine even in strong acetic acid solution. The same effect is thus obtained as in titration of arsphenamine.

The results obtained by titrations of neoarsphenamine in solutions of various acidities are tabulated below.

ACTION OF HYDROCHLORIC ACID ON NEOARSPHENAMINES

Our observations on the effect of hydrochloric acid alone on neoarsphenamines were made in an attempt to precipitate the free neosulphinic acid from cooled aqueous solutions of the drug by means of uniform amounts of diluted hydrochloric acid, and analyzing the precipitates for sulphur and arsenic. The precipitates of neosulphinic acid were filtered on weighed Gooch crucibles using a layer of cotton over asbestos pulp as a filtering medium. After washing with dilute hydrochloric acid, and then cold distilled water, the neo-

TABLE IV

ANALYTICAL DATA OF NEOSULPHINIC ACIDS OBTAINED BY PRECIPITATION WITH HCl

SAMPLE OF NEOARSPHENAMINE	AMT. OF SAMPLE	% AS IN SAMPLE	% S IN SAMPLE	WT. OF FREE NEOSULPHINIC ACID IN GMS.	RATIO OF NEOARS. TO SULPHINIC*	% AS IN NEOSULPHINIC ACID**	% S IN NEOSULPHINIC ACID†	AS TO S RATIO IN NEOSULPHINIC ACID‡	REMARKS
H	0.4	19.35	8.00	0.2275	1.75:1	31.11	7.04	4.43:1	Sample dissolved in 30 c.c. 10% NaCl sol. and precipitated by 2 c.c. 18% HCl. Final acidity = 1.2% HCl. H_2O washings contained As tested by Lehmann's method, but amount not calculated.
J	0.4	18.80	10.34	0.2434	1.64:1	30.11	7.19	4.19:1	Precipitation incomplete. Considerable As in filtrate.
K	0.4	18.87	10 04	0.1987	2.01:1	30.96	6.51	4.75:1	Precipitation incomplete; more acid required for complete precipitation.
L	0.4	20.12	8.87	0.2329	1.72:1	31.88	6.44	4.45:1	H_2O washings tested quantitatively contained 3.7 mg. As; = 11 mg. neosulphinic acid.
M	0.4	18.52	10.89	0.2025	1.97:1	32.89	7.50	4.39:1	Required 10 c.c. 18% HCl to precipitate completely neosulphinic acid. Final acidity = 4.5% HCl.
G	0.4	19.65	9.01	0.2175	1.84:1	33.20	6.55	5.06:1	3.0 c.c. HCl required for complete precipitation.

*The high ratio of neoarsphenamine to neosulphinic acid is due to fact that commercial neoarsphenamines contain only approximately 60 per cent actual organic arsenical component.

**The theoretical As in pure monosubstituted neosulphinic acid is 33.80 per cent.

†The theoretical S in pure monosubstituted neosulphinic acid is 7.22 per cent.

‡The theoretical As to S ratio in monosubstituted neosulphinic acid is 4.68:1.

SUMMARY

Previous methods described for the determination of the chemical composition of neoarsphenamine are either too involved to be of practical importance or are subject to considerable error, because of the fact that they are based on titrations of the drug with iodine in acid solutions and separation of the active organic arsenic constituents from the uncombined organic and inorganic sulphur compounds by precipitation of the former with hydrochloric acid. Organic trivalent arsenic compounds cannot be titrated successfully by iodine in the presence of excess mineral acid, because of a reversible reaction taking place during the titration, this reversible reaction being influenced by increase in acidity.

The combined formaldehyde sulphoxylate groups in neoarsphenamine are either oxidized by iodine in solutions of weak acidity to the corresponding formaldehyde bisulphite groups, or are progressively hydrolyzed in increasing strengths of acid and finally oxidized completely to formaldehyde and sulphuric acid. Neoarsphenamine cannot be precipitated from its solutions by means of hydrochloric acid with any degree of analytical exactness because of the tendency of the precipitated neosulphinic acid to undergo hydrolysis with the formation of arsphenamine hydrochloride and organic and inorganic sulphur compounds, thus increasing the sulphur content of the filtrates.

CONCLUSIONS

1. A reversible reaction takes place when arseno compounds are titrated with iodine in presence of a mineral acid, less iodine being required than is theoretically necessary. The extent of this reversible reaction is directly proportional to the increase in acidity of the titrated solution.

2. Organic acids also have a tendency to promote this reversible reaction, but to a much smaller degree than mineral acids.

3. The combined formaldehyde sulphoxylate group in neoarsphenamine is oxidized by iodine in neutral solution to the corresponding formaldehyde bisulphite group, requiring 2 atoms of iodine.

4. In presence of even dilute solutions of hydrochloric acid, the combined formaldehyde sulphoxylate group undergoes a certain amount of hydrolysis, thus requiring more than 2 atoms of iodine for this group. For complete oxidation of this group 4 atoms of iodine or 2 of oxygen are required.

5. Strong solutions of hydrochloric acid (10 per cent or over) will completely hydrolyze neoarsphenamine into arsphenamine and the decomposition products of formaldehyde sulphoxylate. Arsphenamine hydrochloride has actually been isolated from this reaction.

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THE INFLUENCE OF MAGNESIUM SULPHATE ON THE EXPULSION OF BILE FROM THE GALL BLADDER*

By W. Horsley Gantt, M.D., Baltimore, and G. v. Volborth, M.D., Petrograd

Magnesium sulphate has been widely used during the past six years to cause expulsion of bile into the duodenum. Upon this alleged property of the salt rests its reputation in the drainage of the gall bladder by the Meltzer-Lyon method.[1, 2]

However the subsequent work of some clinicians who have applied this method in the treatment and diagnosis of gall bladder conditions and of several physiologists have not substantiated the original claims made for magnesium sulphate as a stimulator for the contraction of the gall gladder and relaxation of the sphincter of Oddi. (Max Einhorn,[3] Thos. R. Brown, Frazer,[4] J. W. McNee,[5] Bickel.[6]) The marked discrepancy in the views of prominent clinicians, coupled with the fact that one of us (W. H. G.) had had good clinical results (in diagnosis) from this method, led us to make several experiments in the hope of throwing additional light on the action of this salt when introduced into the duodenum.

In 1921 Bassler[14] could not see a contraction of the gall bladder nor obtain bile from the duodenum when magnesium sulphate was introduced through the duodenal tube in anesthetized patients, but the results of Matsuo[16] and of Silverman and Menville[15] are at variance with those of Bassler. Matsuo saw in the duodenum an appearance of dye which he injected into the gall bladder at the same time that magnesium sulphate was given, and Silverman and Menville using a fluoroscope and visualizing the gall bladder by the injection of phenoltetrabromphthalein into the blood, saw the gall bladder become smaller after the administration of $MgSO_4$. Friedenwald, Martindale, and Kearney[13] performed experiments which did not confirm the positive clinical reports, noted above.

In our experiments we tried to have the conditions as near to normal as possible, concerning both the physical condition of the dog and the nerve connections and the condition of the duodenum and of the papilla of Vater, as it is upon this part that the magnesium sulphate is supposed to act. For this reason we did not use the choledochus fistula operation of Prof. Pavlov[7] in which a section of the duodenum surrounding the papilla is excised and sutured to the skin; nor the double biliary fistula as performed by one of us (G.v.V.) previously.[8] We selected the gall bladder fistula of Schiff,[9] because the connections of the papilla of Vater are not disturbed, and the choledochus is pre-

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served intact in this operation. One of us has shown[10] that in a dog with such a fistula, the beginning of the expulsion of bile into the duodenum can be clearly recognized, that the expulsion of bile through the gall bladder is at the same time inhibited, and that within ten to fifteen minutes after the bile begins to flow into the duodenum, there appears in the gall bladder a pure colorless mucus, without a suggestion of bile. With the cessation of the flow of bile into the duodenum, i.e., at the end of the period of digestion, bile reappears in the gall bladder, and the mucus flowing from the gall bladder fistula becomes suddenly colored.

In addition to the gall bladder fistula of Schiff we performed in our dogs a lateral duodenal fistula, according to Pavlov.[7] Without disturbing the continuity of the duodenum, it was sutured to the surface of the abdomen, so that the opening in the duodenum was about 7 to 9 cm. below the papilla of Vater. This operation is to a certain degree a substitute for the choledochus fistula in the double fistula operation.[8] The arrangement of the duodenal fistula (close to the papilla of Vater) permits us to introduce the magnesium sulphate directly into the duodenum, as was done by Meltzer,[1] so that the possibility of the action on the biliary apparatus is insured. And though we cannot quantitatively determine the amount of bile in the duodenum, we can make certain at any moment whether it is flowing into the duodenum, for we can at any time obtain a specimen of the duodenal contents through this fistula.

The following experiments in dogs were not carried out until a month had elapsed after the operation, and we had assured ourselves that the condition of the animals and of the biliary secretions were normal. The experiments were performed in the fasting condition, fifteen to twenty-four hours after the last feeding, and according to the general conditions of experimentation in Pavlov's laboratories.[7]

Fifty c.c. of a 25 to 33 per cent solution of magnesium sulphate at a temperature of 35-40° C. was introduced into the duodenal fistula, and after ten to fifteen minutes, the duodenal fistula was opened and the solution allowed to run out.

We append here protocols from two of our dogs. Table I shows the influence of magnesium sulphate upon the volume of bile secreted (the fluid from each fistula being collected and measured every fifteen minutes). Table II shows the influence of magnesium sulphate on the pressure of bile in the biliary system.

The objection can be raised that conditions are not normal when there is a gall bladder fistula because the bile continuously flowing out of the fistula may prevent a filling of the gall bladder and ducts, and secondly in such a case the magnesium sulphate could not exert its influence to cause expulsion of the bile into the duodenum, because the gall bladder would contract to force the bile along the line of least resistance, i.e., through the wide fistula instead of along the smaller cystic duct. Although this objection was partly refuted through an earlier experiment by one of us,[8] we devised here a special arrangement of the apparatus to prevent the flow of bile from the gall bladder fistula and to maintain a constant and normal pressure in the biliary system.

A schematic representation of the operation and apparatus is shown in Fig. 1.

TABLE I

DOG 1, "JACK". APRIL 10, 1923

TIME	GALL BLADDER FISTULA		DUODENAL FISTULA
11:15			
11:30	2.8 c.c.	All specimens = pure bile.	Little or no flow into the duodenum.
12:45	2.2		
12:00	2.6	Amt. secreted for 1st hr. (12:00-1:00) = 4.6 c.c.	
11:45	0.9		Reaction weakly acid.
12:30	0.5		
12:15	0.6		
12:50§		§Introduction of 20.0 c.c. 30% $MgSO_4$ through the duodenal fistula, and allowed to remain 10 min.	
1:05	1.8	All specimens pure bile as formerly.	Fistula opened at 12:40 P. M.: 5.0 c.c. clear fluid, without bile obtained; reaction weakly acid.
1:20	0.8		
1:35	1.2		
1:50	0.6	Amt. excreted for 2nd hr. (1:05-2:05) = 4.4 c.c.	
2:05	1.0		
		During the whole experiment there was practically no flow into the duodenum.	

DOG 1, "JACK". APRIL 13, 1923

TIME	GALL BLADDER FISTULA		DUODENAL FISTULA
9:45			
10:00	0.4 c.c.	Pure mucus.	
10:07		Pure bile begins to flow and continues till end of experiment.	
10:15	4.8		
10:30	4.2		
10:45	4.6	Amt. secreted for 1st hour. (10:00-11:00) = 17.4 c.c.	
11:00	2.8		
11:00§		§Introduction 50.0 c.c. 30% $MgSO_4$ into the duodenal fistula and allowed to remain 10 min.	
11:10			Duodenal fistula opened and a few drops of clear fluid without bile obtained.
11:15	3.1	All specimens pure bile.	
11:30	2.2		After 11:00 A. M. there was a flow of clear fluid without bile; reaction neutral.
11:45	1.71	Amt. excreted for the hr. (11:00-12:00) = 8.1 c.c.	
12:00	1.1		
12:15	1.1		

DOG 1, "JACK". APRIL 14, 1923

TIME	GALL BLADDER FISTULA		DUODENAL FISTULA	
12:00				
12:15	1.6 c.c.	Secreted ¾ hr. (12:45-1:15) = 3.8 c.c.	24.0 c.c.	Weakly colored bile.
12:30	2.0		16.0	Reaction acid; no bile.
12:45	2.0		8.0	Idem.
1:00	0.8		4.5	"
1:15	1.0		6.00	Reaction acid; bile colored.
1:15§		§Introduction 50.0 c.c. 25% $MgSO_4$ in duodenal fistula, and allowed to remain 10 minutes.		
1:30	2.3	Secreted for ¾ hr. (1:15-2:00) = 4.5 through g. b. fist.	8.0 c.c.	Reaction acid; no bile.
1:45	1.2		30.0	Idem. " "
2:00	1.0		95.0	" " "

DOG 1, "JACK". APRIL 20, 1923

TIME	GALL BLADDER FISTULA		DUODENAL FISTULA	
10:30				
10:40	1 drop mucus.			Fistula opened; slight amt. bile.
10:45	0.8 c.c.		3.0 c.c.	
11:00		Amt. excreted through the g. b. fist. for the hr. (11:00-12:00) = 6.6 pure bile.		Reaction neutral; strongly bile colored.
11:15	2.0		3.2	
11:30	2.2		11.0	Neutral no bile.
11:45	1.6			" " "
11:45§		§Introduction 50.0 c.c. 25% $MgSO_4$ through duo. fistula and allowed to remain 15 min.		
12:00	1.4		18.5 c.c.	Clear bile free fluid; reaction neutral.
12:15	1.4		3.0	
12:30	1.0		1.0	Reaction alkaline; bile free, for all specimens till end of exp.
12:45	1.1		0.5	
1:00	1.1		0.5	
1:15	1.1		0.5	

TABLE I—CONT'D

DOG 2, "LORA". FEBRUARY 6, 1924

TIME	GALL BLADDER FISTULA		DUODENAL FISTULA	
5:30				
5:45	0.6 c.c.	All specimens pure bile. Amt. from g. b. for the hr. (5:30-6:30) = 1.5 c.c.		
6:00	0.4			
6:15	0.2			
6:30	0.3			
6:30§		§Introduction 50 c.c. 30% $MgSO_4$ through duo. fistula and allowed to run out after 5 min. At 6:35 fistula was opened for $MgSO_4$ to drain out. At 6:40 some alkaline flow of mucus from the duodenal fistula.		
6:40				
6:45	0.8		20.0 c.c.	Bile free fluid, mucoid, all specimens, to end of experiment.
7:00	0.4	All specimens pure bile. Amt. from the g. b. fist. for the hr. (6:30-7:30) = 1.5 c.c.	0.5	
7:15	0.3		0.5	
7:30	0.0		0.0	

TABLE II

EXPERIMENTS ON THE PRESSURE OF BILE IN THE BILIARY SYSTEM, MEASURED IN MM. OF BILE. SEE FIG. 1.

DOG 1, "JACK". APRIL 15, 1924

TIME	BILE PRESSURE IN BILIARY SYSTEM		DUODENAL FISTULA
11:45			
12:00	17. mm.	Rises gradually to 25. mm.	Several drops bile-free fluid.
12:15	25.		
12:15§		§Introduction 50.0 c.c. 30% $MgSO_4$ into the duodenum through the duo. fistula.	
12:30	24-38	Sinks to 24 mm., and then rises gradually to 38 mm.; then rises gradually to 46 mm. and becomes stationary at 51 mm.	
12:45	46.		Fistula opened; 10 c.c. of a cloudy alkaline fluid obtained; bile-free.
1:00	51.		At 1:00 P. M. several drops of a clear alkaline, bile-free fluid.

DOG 2, "LORA". FEBRUARY 19, 1924

TIME	BILE PRESSURE IN BILIARY SYSTEM		DUODENAL FISTULA	
11:50	50. mm.			
12:10	50-56	Rises from 50 to 56, and in last min. to 72. Varies between 72 and 74.	4.0 c.c.	Clear, alkaline, bile-free.
12:25	72-74		Several drops,	" " "
12:25§		§Introduction 50 c.c. 30% $MgSO_4$ through duo. fist. and after 7 min. duodenal fist. opened.		
12:40	78-80	Varies 78-80.	13.5 c.c.	Cloudy, bile-free fluid.
12:55	80-82	" 80-82.	18.5	Clear, alk., " " "

DOG 2, "LORA". FEBRUARY 11, 1924

TIME	BILE PRESSURE IN BILIARY SYSTEM		DUODENAL FISTULA
5:00	22 mm.		
5:15	22-24		Several drops of mucus.
5:30	24-28		" " clear, bile-free fluid.
5:30§		§Introduction 70 c.c. 30% $MgSO_4$ through the duodenal fistula; fist. opened in 15 min.	
5:45	28-36		Reaction alkaline; bile-free.
6:00	36-46		" " " "
6:15	44.		" " " "

DOG 2, "LORA". FEBRUARY 13, 1924

TIME	BILE PRESSURE IN BILIARY SYSTEM		DUODENAL FISTULA	
5:30	80.00 mm.			
5:45	80-84		9.0 c.c.	Clear, bile-free fluid.
5:45§		§Introduction 40 c.c. 25% $MgSO_4$ through the duodenal fistula; fistula opened after 10 min.		
6:00	84-80		10.0 c.c.	Neutral, bile-free fluid.
6:15	82-84		1.0	Alkaline, bile-free.
6:30	80		1.0	" " "

VARIATIONS IN THE BLOOD-SUGAR CONTENT FOLLOWING THE ADMINISTRATION OF INSULIN*

By Henry J. John, M.D., Cleveland Clinic, Cleveland, Oh.

PROLONGED and repeated observations of the effects of the intravenous injection of insulin on blood-sugar content have shown unexpected and marked variations not only in different patients, but even in the same patient after repeated doses. These variations have been so contrary to my own preconception of the action of insulin that it has seemed worth while to present the observations in a group of individual cases. It is certainly important to determine if possible upon what factors the individual response to insulin depends; and this can only be determined by accumulated experience in large numbers of cases.

Graphic charts present the best material for comparative study, and I am therefore presenting two series of charts, one showing the effects of repeated doses of insulin in 20 patients during a period of seven hours (Charts 1 and 2), each of the other nine charts showing the effects of insulin in a single patient on two or more days (Charts 3 to 11).[1]

In every case the observation period started at about 8:30 A.M. (indicated as 0 hr.), the first dose of insulin being followed by breakfast; lunch was taken at 11:30 A.M.—3 hours; and dinner at 4:30 P.M.—7 hours. The diet was uniform, excepting when otherwise noted on the chart, and consisted of 100 gm. of carbohydrate, 60 gm. protein, 128 gm. fat, 1800 calories. The doses of insulin varied from 3 to 60 units. In interpreting the charts, the effect of the meals on the blood sugar must be taken into consideration as they are always followed by a physiologic postprandial hyperglycemia, which with but few exceptions is exaggerated in diabetes.

A study of the 20 individual curves included in Charts 1 and 2 will show that in all but four (117672, 130990, 114782, 136108) an immediate drop in the blood-sugar content followed closely after the primary dose of insulin. With but one exception the drop is moderate but in that one case—133929—the drop amounted to 281 mg./100 c.c. during the first hour.

In one of the four cases, in which an actual rise of the blood sugar occurred immediately after the administration of the primary dose of 20 units of insulin, the rise continued even after the administration of 10 more units of insulin two hours later.

Chart 3 is that of a fairly severe case of diabetes in a young man twenty-six years of age. On April 13 following the afternoon administration of 16

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[1]*Explanation of the charts:* The heavy lines represent the successive changes in the blood-sugar level, as indicated by the measurements made at the points specified by the heavy dots intersecting the lines. The dosage of insulin in units—and the time of administration—is indicated by the numbers in the circles. The blood sugar scale—in mg. per 100 c.c.—is indicated at the left margin. The blood sugar estimations were made with the Myers' modification of the Benedict method.

units of insulin, there was a steady drop of the blood sugar which was estimated every fifteen minutes for the next three hours, 30 units of insulin given an hour later being followed by a much less marked drop down to 52-56 mg./100 c.c. where the blood-sugar level was maintained with only a slight reaction forty-five minutes after the second dose. On April 24, however, the afternoon dose of 15 units was followed by a rise of the blood sugar as indicated on the chart.

Chart 4 is that of a young woman twenty-five years of age who was brought to the hospital in coma. This picture therefore is not complicated by the taking of any food. As is shown by the chart, on August 21 following the first dose of 7 units of insulin, the blood sugar rose for two hours and twenty

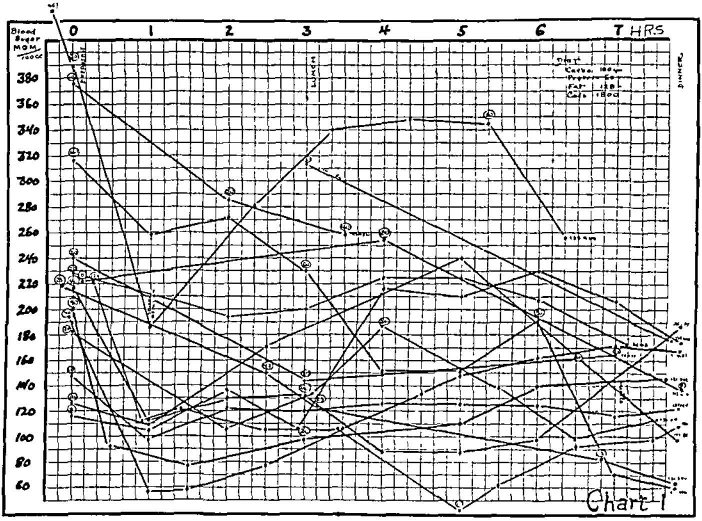

Chart 1.—Blood-sugar curves showing variations in the response to varying doses of insulin in sixteen diabetic patients.

minutes, when the administration of 15 more units was followed by a moderate decrease of the blood sugar which was progressive for four hours, when the administration of 16 more units was followed by a more marked drop. Two days later the administration of the morning dose of insulin—15 units—was followed by a drop of the blood sugar from 297 to 232 mg./100 c.c. at the end of three hours; 15 more units were then given following which the blood sugar rose to 375 mg./100 c.c.; when 26 more units of insulin reduced the blood sugar to 263 mg./100 c.c. in three hours and twenty minutes.

Chart 5 is that of a woman thirty-nine years of age, a severe diabetic. On August 28, 1924, the administration of 25 units of insulin in the morning was followed by a rise of blood sugar from 300 to 348 mg./100 c.c., when 25 units more were administered following which there was a steady and continued drop

of the blood sugar to 141 mg./100 c.c. On February 3, 1925, the blood-sugar level in this same patient was 384 mg./100 c.c. At this time the administration of 25 units of insulin was followed in one and one-half hours by a drop to

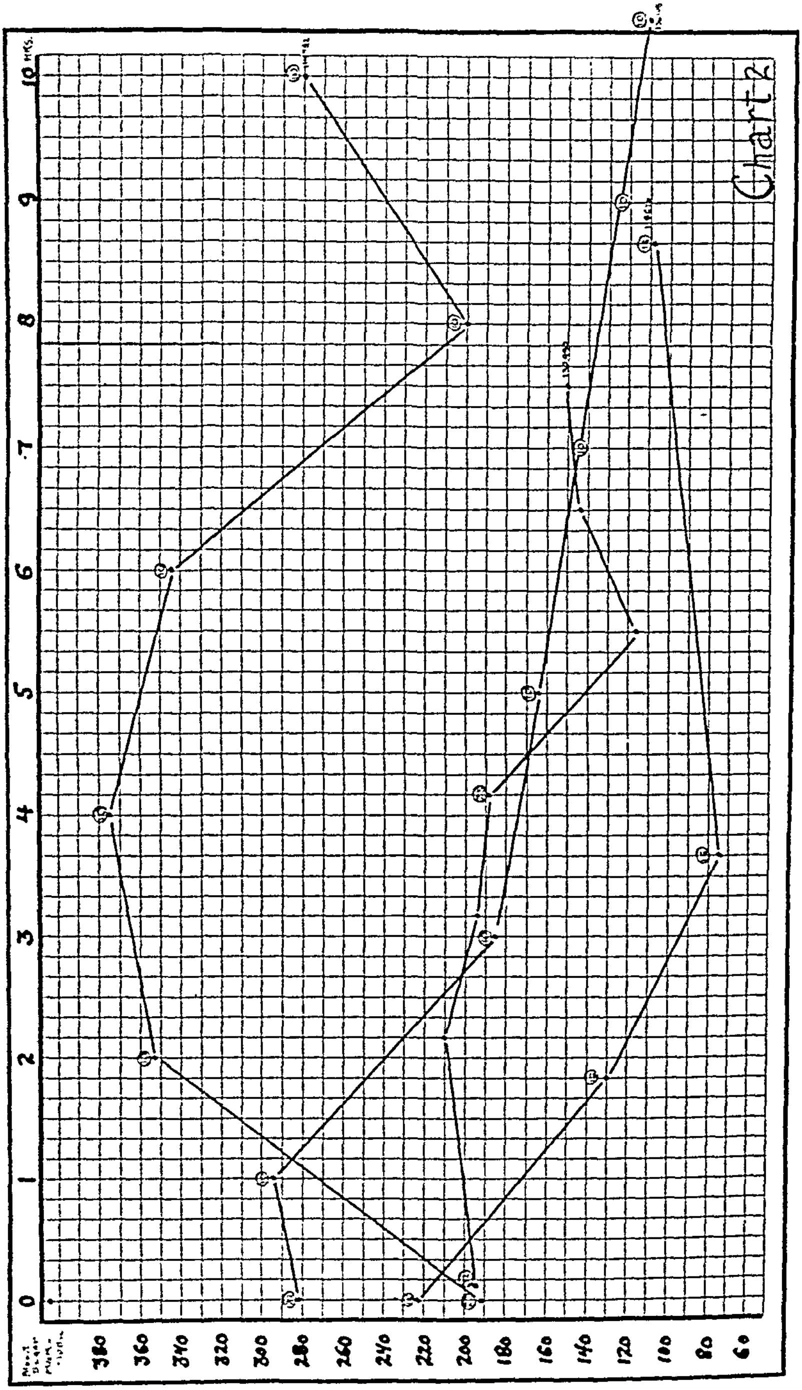

Chart 2.—Blood-sugar curves showing variations in the response to varying doses of insulin in four diabetic patients.

303 mg./100 c.c.; after this during the next two hours the blood sugar rose to 336 mg./100 c.c. when again 25 units of insulin were given, with a resultant drop during the next two and one-half hours to 171 mg./100 c.c. At this point

the blood sugar again began to rise reaching 275 mg./100 c.c. in two hours. One might be tempted to explain the rise in the second curve by the assumption that the insulin effect wore out at the end of two and one-half hours; but how can one explain the primary rise in the first curve? One might assume that the primary dose of insulin was not sufficient to counteract the postprandial

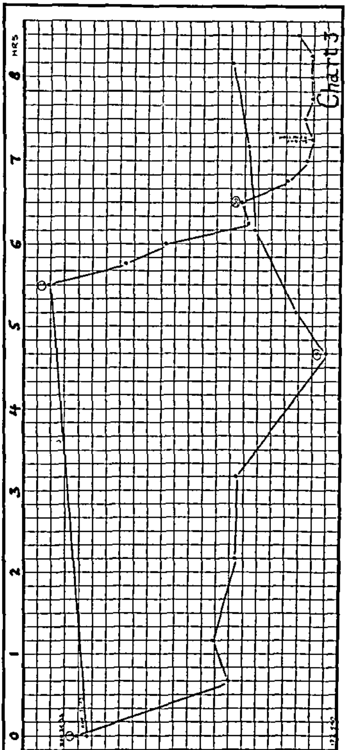

Chart 3.—Blood-sugar curves showing variations in the response to insulin in a diabetic patient on two different days.

hyperglycemia—but certainly 25 units of insulin should be sufficient to counteract the 20 grams of carbohydrate included in the food taken at breakfast.

Chart 6 is that of a man twenty-seven years of age. On November 18, 1924, a rise in the blood sugar from 190 to 263 mg./100 c.c. followed breakfast. One hour after breakfast the administration of 10 units of insulin was followed by a steady fall of the blood sugar to 105 mg./100 c.c., six hours later, when the

patient had a questionable reaction. Two days later, on November 20, 10 units of insulin were given at noon, when the blood sugar was 146 mg./100 c.c., following which during the next four hours there was a steady drop to 112 mg./

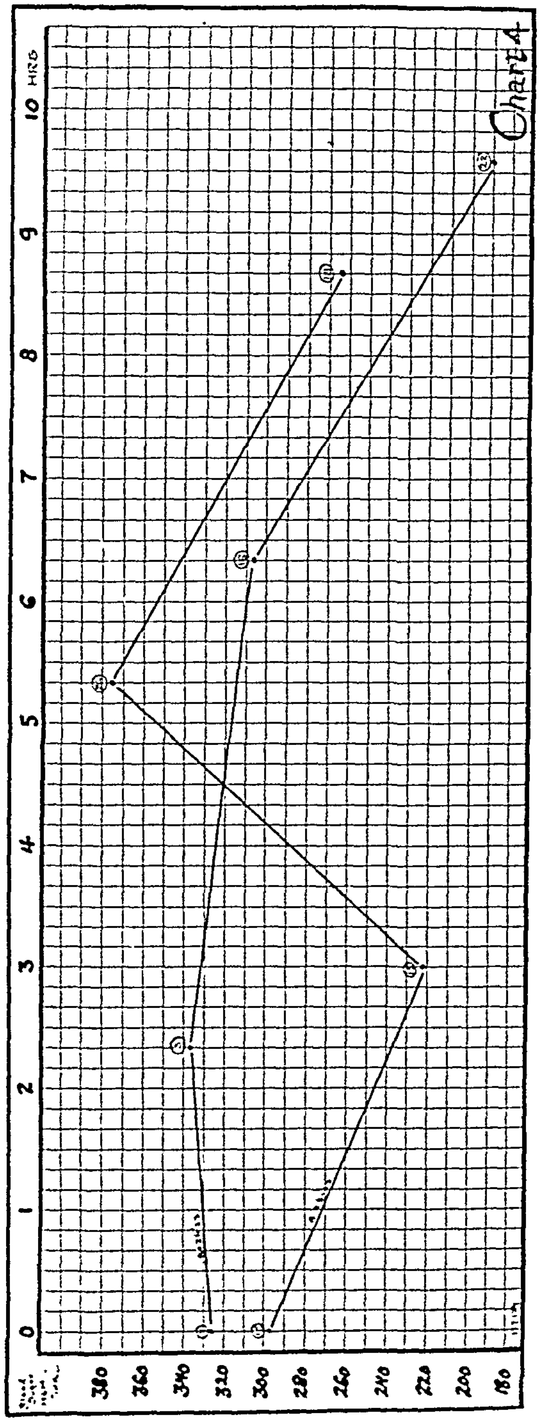

Chart 1.—Blood-sugar curves showing variations in the response to insulin in a diabetic patient on two different days.

100 c.c., the progressive decrease in the blood sugar being uninterrupted by luncheon, which was taken immediately after the dose of insulin was given. In contrast to these two curves is that of the following day when with a fasting

blood sugar of 92 mg./100 c.c. the administration of 10 units of insulin was followed by a rise throughout the day to 217 mg./100 c.c. seven hours later.

Chart 7 gives observations during nine successive days on a young man twenty-six years of age who was brought to the hospital in coma. On admission the CO_2 tension in the blood was 21.4 volume per cent; there was heavy acetone in the plasma, and marked hyperglycemia. On the morning of February 14,

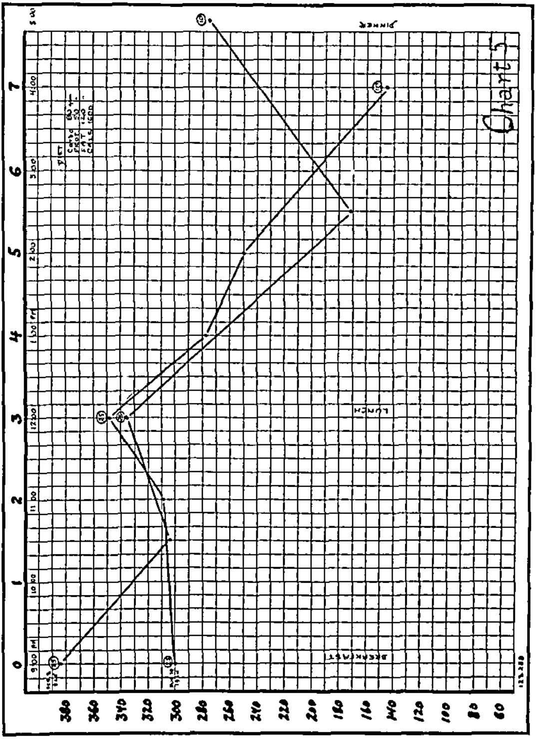

Chart 5.—Blood-sugar curves showing variations in the response to insulin in a diabetic patient on two different days.

1925, following the administration of 20 units of insulin, the blood sugar dropped from 280 to 256 mg./100 c.c. in an hour and ten minutes, when 40 more units of insulin were administered together with the intravenous injection of 250 c.c. of a 10 per cent glucose solution. This was followed by an increase of the blood sugar to 316 mg./100 c.c. during the next hour and a half, when another dose of 40 units of insulin together with 250 c.c. of the 10 per cent glucose solution was followed by a further rise to 352 mg./100 c.c. Again 40 units of insulin with another 250 c.c. of 10 per cent glucose solution were given and

two hours later 20 units of insulin were given hypodermically with a resultant decrease of the blood sugar during the next six hours to 120 mg./100 c.c., when 40 units of insulin with glucose solution were again given with no resultant reaction. On the following day, February 15, the patient started with a blood sugar content of 442 mg./100 c.c.; as indicated by the chart repeated doses of insulin were required to reduce the blood sugar to 187 mg./100 c.c. at the end of six hours with later decreases after two more successive doses to 53 mg./100 c.c. It is of interest to note that although two of these doses of insulin were combined with 250 c.c. of 10 per cent glucose, the fall in blood sugar was continuous.

On February 16, the morning blood-sugar level was only 200 mg./100 c.c. The administration of 40 units of insulin was followed by a slight drop to 172 mg./100 c.c. Two hours later, when 40 units of insulin together with 250 c.c. of glucose solution were given with a subsequent very slight rise during

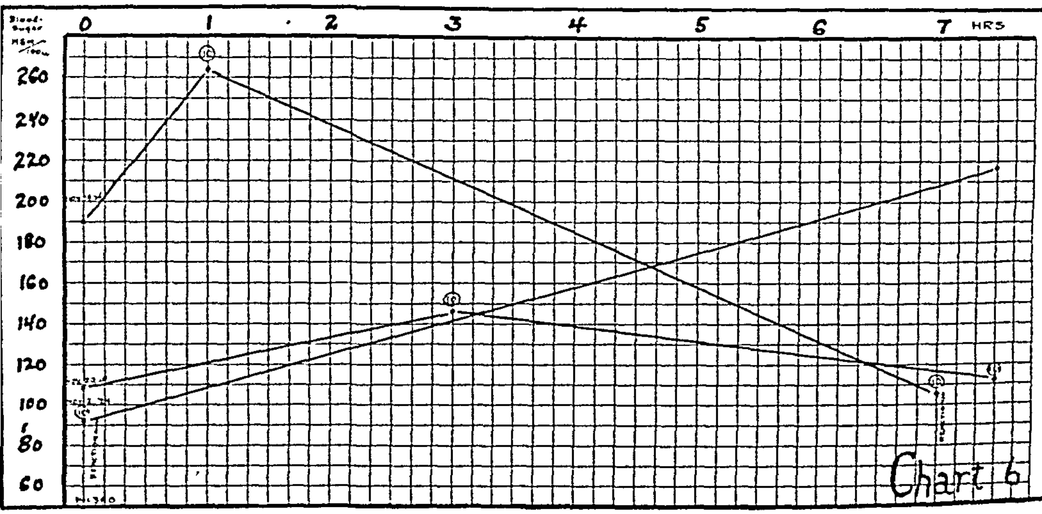

Chart 6.—Blood-sugar curves showing variations in the response to insulin in a diabetic patient on three different days.

the next two hours to 175 mg./100 c.c. Twenty units of insulin at this point were followed by a drop to 85 mg./100 c.c.; and two further doses of 20 units each carried the blood sugar to 67 mg./100 c.c. during the next four hours.

The curves of the following days give us further graphic pictures of the variability of the blood-sugar changes following the administration of insulin. The curves of February 18 and 19 are especially interesting because of the small doses of insulin which were given.

Chart 8 is that of a young man thirty-five years of age, with severe diabetes, and generalized xanthoma diabeticorum.

Chart 9 is that of a woman thirty-four years of age who was brought to the hospital in coma. In each of these the progress of events is sufficiently noted by the charts.

Chart 10 gives graphically the observations on seven successive days in a very severe case of diabetes in a woman thirty-eight years of age. The individual curves in this case show a considerable variation. The food was limited

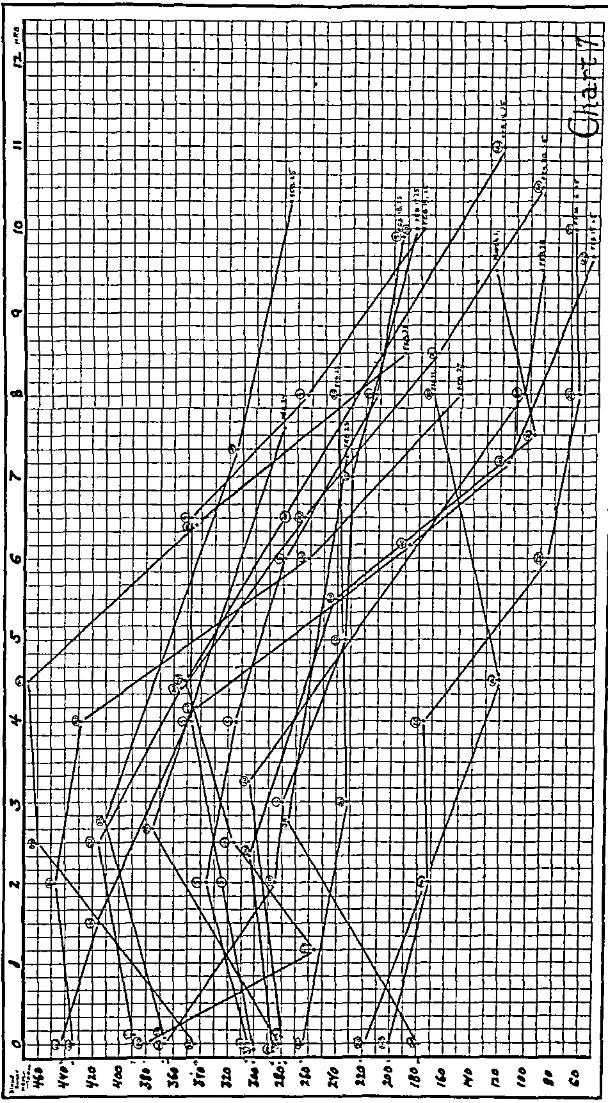

Chart 7.—Blood-sugar curves showing variations in the response to insulin in a diabetic patient on nine successive days.

to practically a maintenance diet. On four mornings a drop in the blood sugar followed the first administration of insulin; on the other mornings the primary dose of insulin was followed by a rise in the blood sugar. In every instance, however, the blood sugar decreased after the dose given at noon. The more

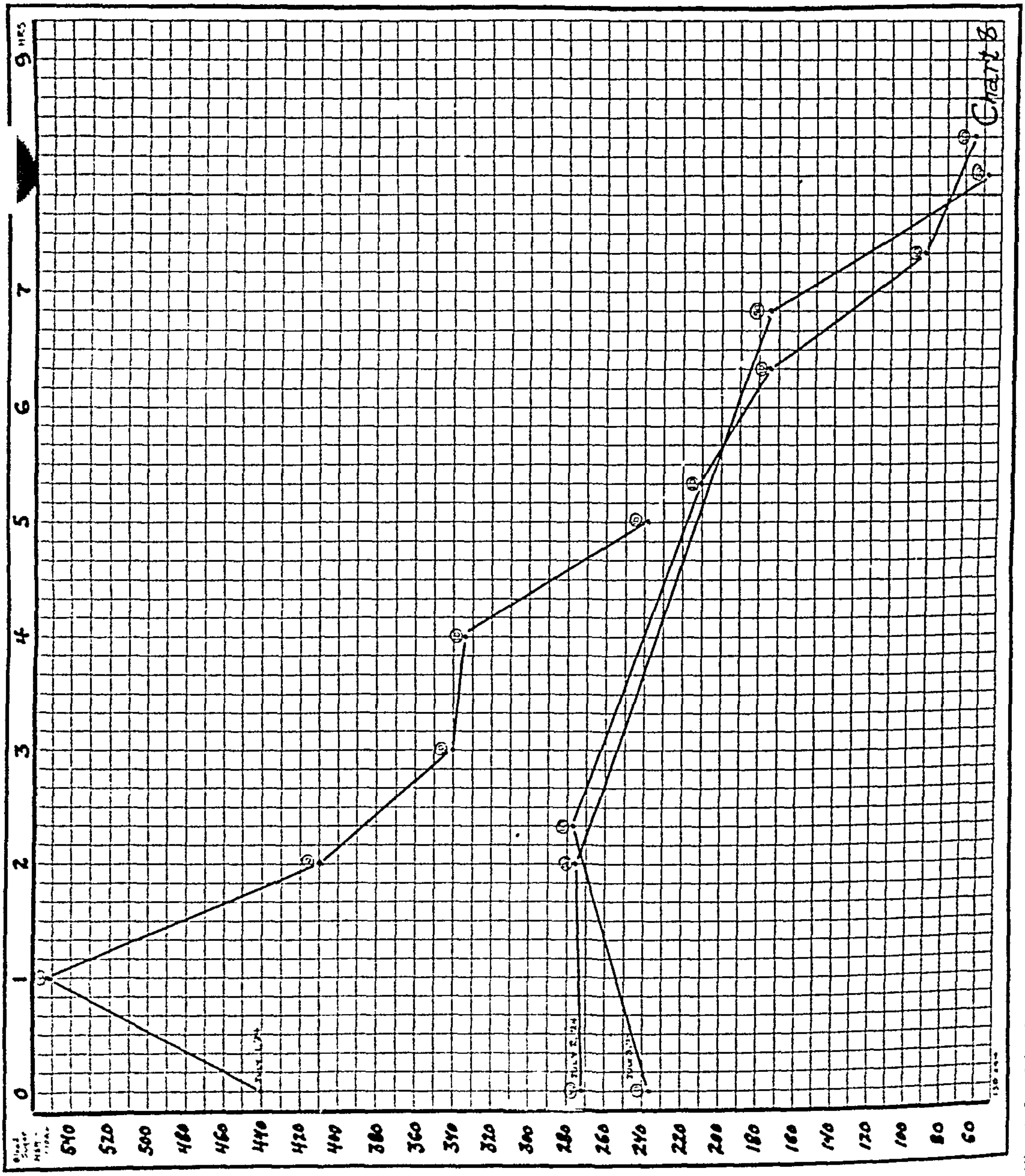

Chart 8.—Blood-sugar curves showing variations in the response to insulin in a diabetic patient on three successive days.

marked response during the afternoon would seem to suggest that insulin may have a cumulative action. This, however, would not explain the apparent lack of relation between the size of the dose and the degree of response.

Chart 11 is presented as it seems to summarize well the indications of those previously analyzed, and presents in brief the extent of our present knowledge regarding the practical value of insulin in cases of severe diabetes. The patient

was a woman forty-eight years of age who was in coma when I first saw her. Hourly doses of insulin were at once instituted and hourly estimations of the blood sugar were made throughout two successive days.

Although the insulin dosage was uniform and given at regular intervals,

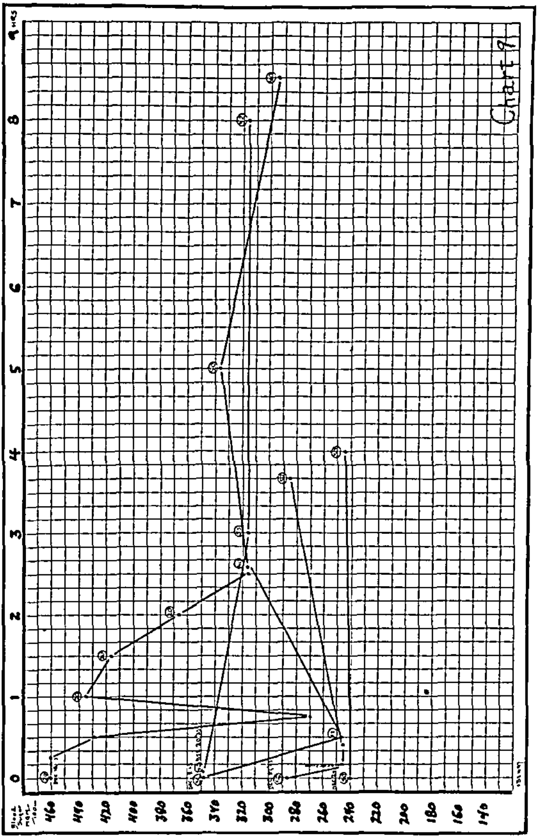

Chart 9.—Blood-sugar curves showing variations in the response to insulin in a diabetic patient on five different days.

the response during the first nine hours was not uniform. Nevertheless, during the first day with the exception of the temporary slight increases of the blood sugar there was a fairly uniform drop to below the normal level; and during the second day, this low level was maintained by means of the administration of 20 units of insulin at regular intervals.

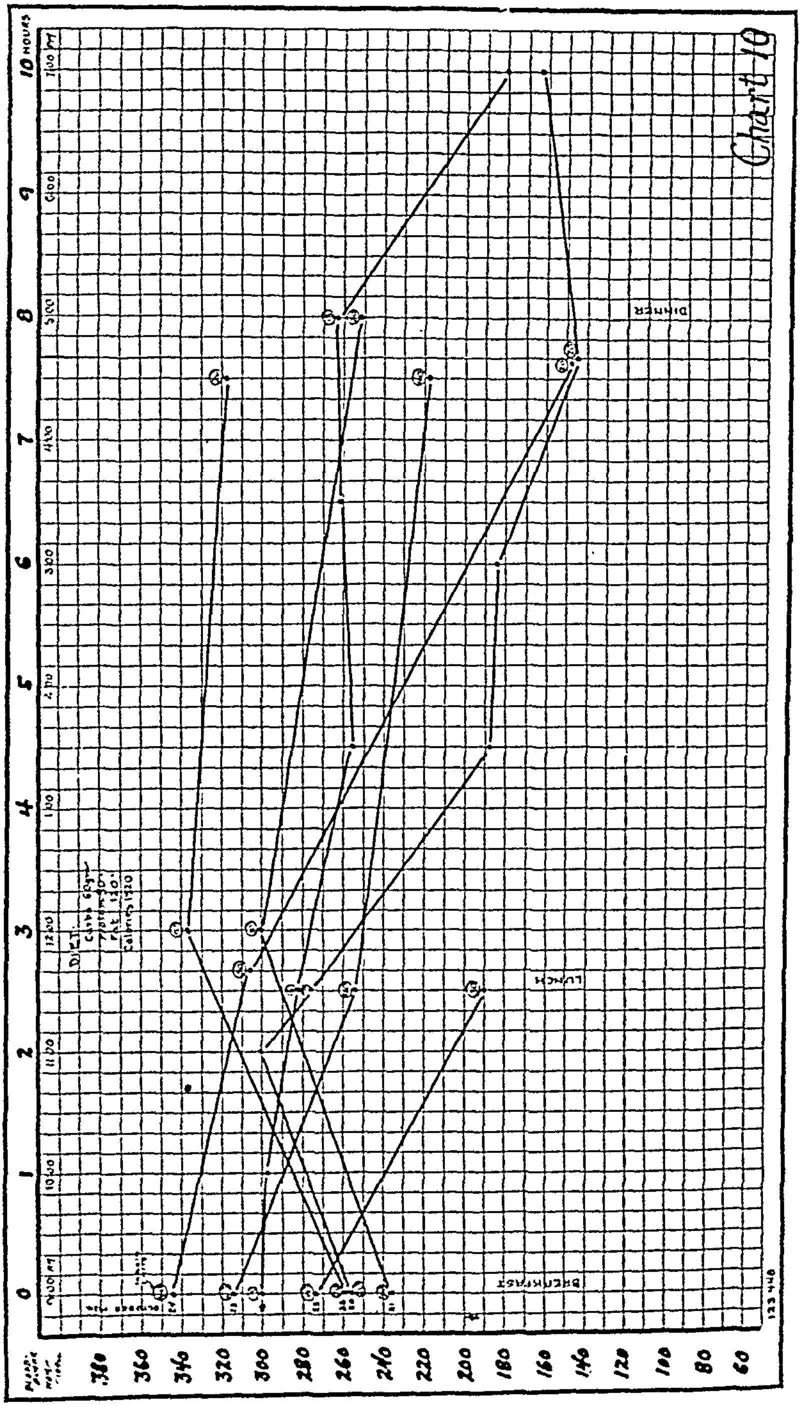

Chart 10.—Blood-sugar curves showing variations in the response to insulin in a diabetic patient on seven successive days.

The outstanding features of the action of insulin in this case were the following: (1) A fairly uniform decrease in the blood sugar from a very high level; (2) a very slight decrease in the blood sugar when it was at or slightly below the normal level; (3) no change in the blood sugar after a hypoglycemic level has been reached; (4) no reactions when blood sugar was as low as 42 mg./100 c.c.

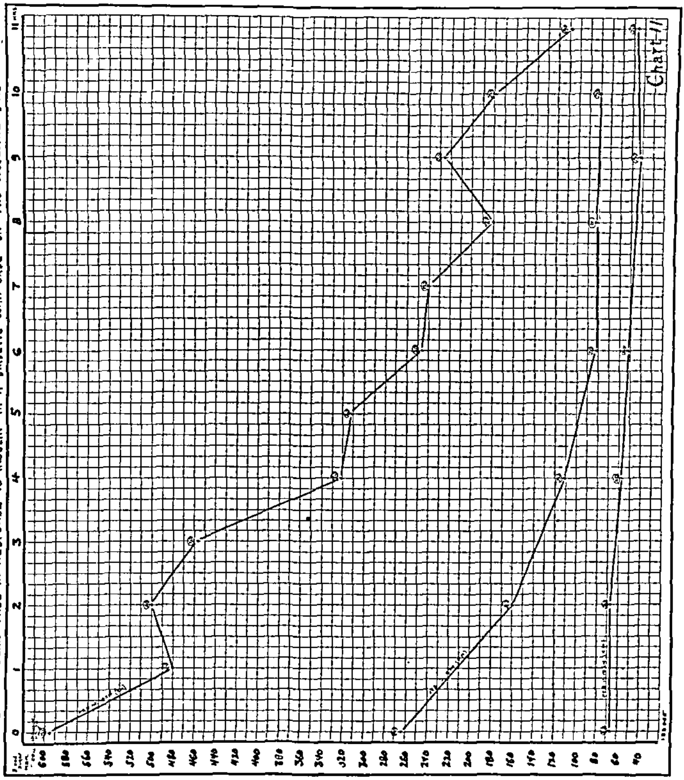

Chart 11.—Blood-sugar curves showing variations in the response to insulin in a diabetic patient on two successive days.

CONCLUSIONS

1. The amount of insulin administered intravenously appears to bear no regular relation to the fall of blood sugar.

2. While the administration of insulin usually produces a decrease in the blood sugar, in some instances it is followed by an increase which may or may not be related to postprandial hyperglycemia.

3. In general, whatever the primary effect of insulin, repeated doses result in a decrease in the blood sugar; this apparently cumulative effect, however, is independent of the size of the doses.

4. The level of blood sugar per se is not a criterion of the probability that a reaction will follow the administration of insulin. Thus a large dose of insulin may be given in the presence of hypoglycemia without any resultant reaction; and in another case a reaction may occur in the presence of a marked hyperglycemia.

A PROBLEM IN INSULIN THERAPY*

By William J. Mallory, A.M., M.D., and Joseph H. Roe, Ph.D., Washington, D. C.

THE general principles underlying the management of diabetes are now well established and accepted.

One of these principles is individualization. Each patient presents a peculiar problem.

Diet must be given with accuracy, according to the special requirements and limitations of the patient, and, when the necessary diet cannot be metabolized, insulin must be given.

The dose of insulin must be quantitatively adjusted to the patient's tolerance deficiency. A dose too small is bad, one too large is worse. Not only must the dose be adequate and accurate in amount, but it must be given at the physiologic moment.

From the beginning of the use of insulin, it has been known that the dose must be administered just before meals, in order that it may meet the metabolic requirements during the absorption of food from the digestive tract.

That it may be required also after the absorption period; (except in coma, and during surgical operations) is not so generally known, but is well illustrated by the following case.

Diabetes was recognized in J. W. at the age of six, two years ago. He complained of feeling tired; there was thirst, polyuria, and 6 per cent of sugar in the urine.

Dieting produced little relief, and he was taken to a hospital, where he contracted scarlet fever.

There was no gain in weight for nineteen weeks.

Insulin was then given, up to nine units twice a day, with an increase of weight from 44 to 52 pounds.

On July 24, 1924, he fell and fractured the right ulna. Two days later he was admitted to the George Washington University Hospital in diabetic coma, from which he promptly recovered under insulin treatment.

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*Read before the Medical Society of the District of Columbia, May 13, 1925.
From George Washington University.

The fracture was reduced, and, later, infected tonsils removed and abscessed teeth extracted.

From the amount of sugar in the daily twenty-four-hour urine, and the high morning blood-sugar level, it appeared that the patient required increasing doses of insulin.

The dose was, therefore, gradually raised from ten units just before breakfast, lunch, and dinner, to twenty-two units, given at the same periods.

This did not produce the anticipated result. Sugar continued to be excreted, and the morning blood-sugar level remained high, as shown in Chart I. On a day when ten grams of sugar was excreted and the morning blood sugar was 337 mg., there occurred a severe insulin reaction,—and the blood sugar

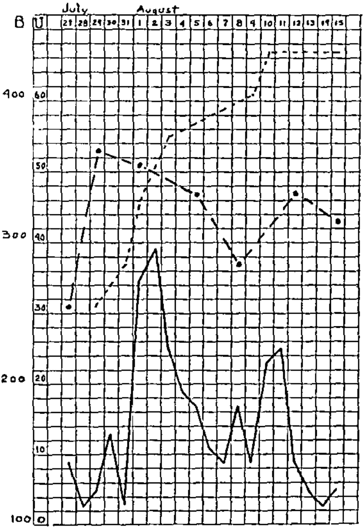

Chart 1.—Sugar excretion in grams, unbroken line, ————; blood sugar in milligrams per 100 c.c. of blood, broken line, - - - - - - - -; insulin in units (U.20), dotted line, ...; observe marked increase in insulin dosage without corresponding decrease in blood sugar and only slight decrease in sugar excretion.

was found to be 52 mg. per 100 c.c. The urine was then collected every three hours for the twenty-four-hour period, and the sugar determined quantitatively in each specimen. The result is shown in Chart 2.

Chart 3 shows the relation between sugar excretion, blood-sugar level, and insulin administration.

The midday dose of insulin was then omitted and 10 units given at 5 A.M. The result is shown in Chart 4.

An effort was then made to distribute the insulin dosage to meet the requirements of the three meals and also to control the hyperglycemia between 12 midnight and 7 A.M.; the result is shown in Chart No. 5.

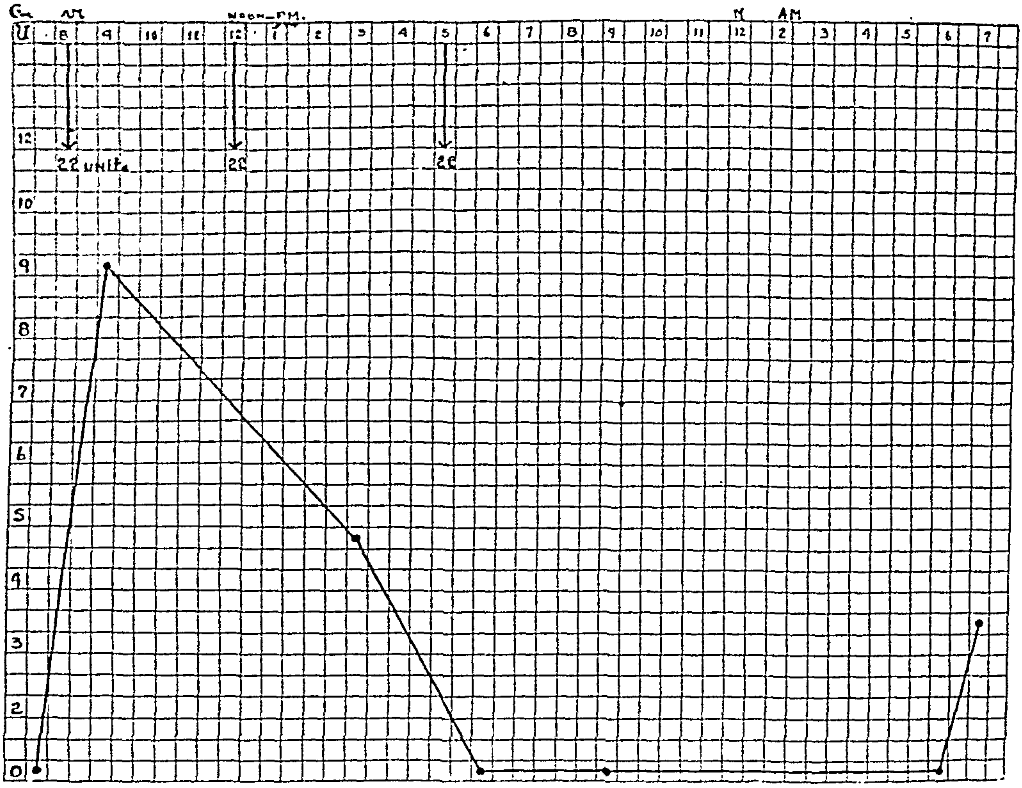

Chart 2.—Observe the excretion of 9 grams of sugar between 7 A.M. and 9 A.M. the gradual decrease to zero at 6 P.M., where it remained till 6 A.M., followed by the excretion of 3.5 grams between 6 A.M. and 7 A.M.

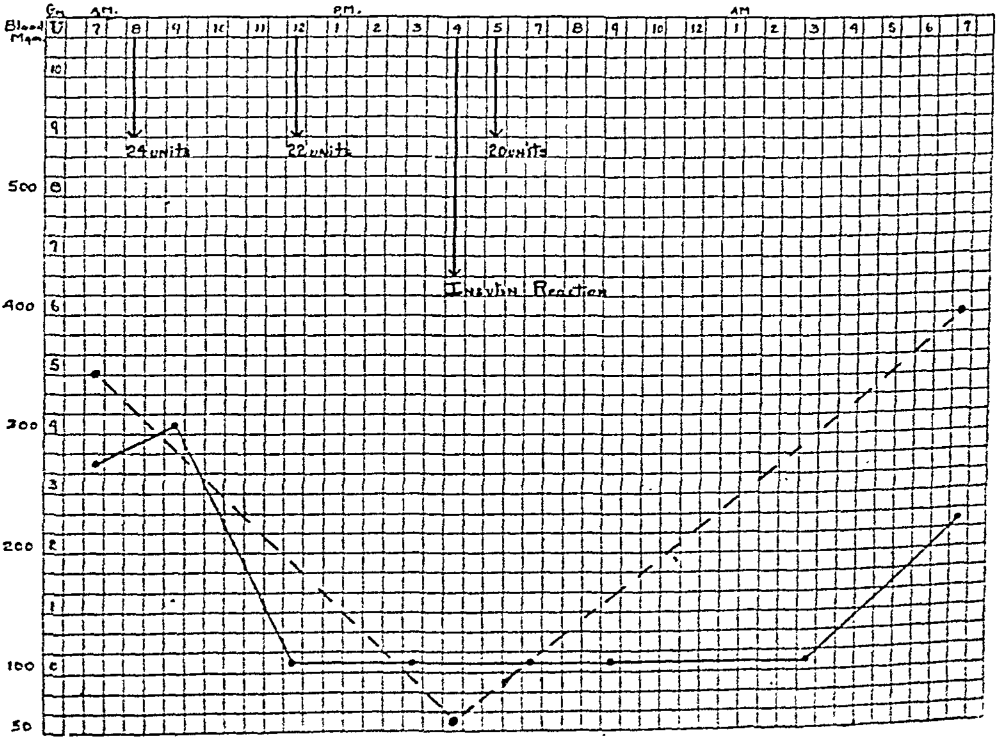

Chart 3.—Observe that while no sugar was excreted between 12 noon and 3 A.M., 2.5 grams were excreted between 3 A.M. and 7 A.M.

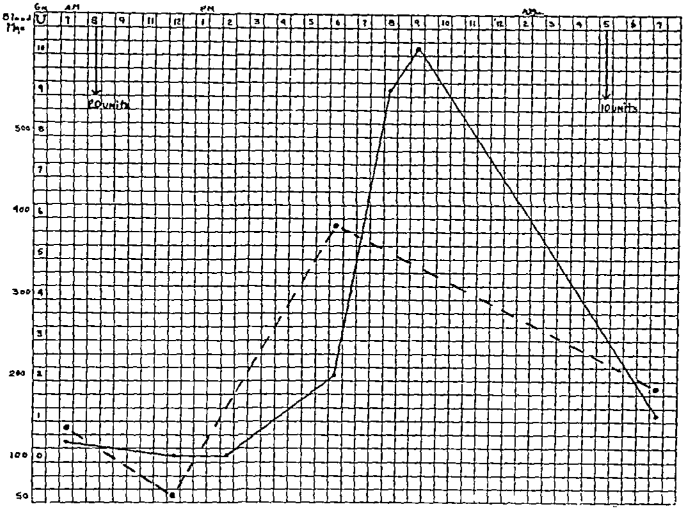

Chart 4.—Observe the effect of the omission of the insulin at noon, and the effect of 10 units at 5 A.M. on the morning blood-sugar level.

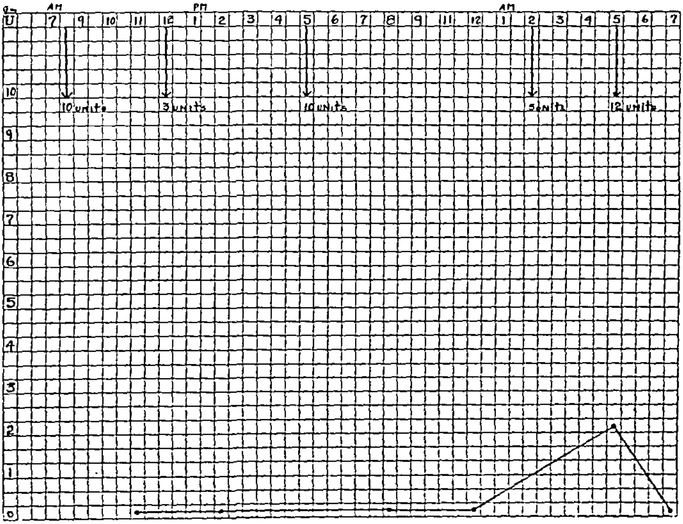

Chart 5.—After redistribution and reduction of insulin dosage to 38 units. Observe the control of sugar excretion till midnight. Two grams of sugar were still excreted between midnight and 5 A.M. Compare with Chart 1, when 64 units were given in three doses.

Two grams of sugar were still excreted some time between midnight and 5 A.M., but there was some improvement, in that the total dose of insulin was reduced by twenty-six units per day and the blood sugar kept at a much lower level than it had been on twenty-two units three times a day. See Chart 6.

A further adjustment in time of administration permitted a reduction of the total daily dosage to thirty-five units, with the result shown in Chart 7.

COMMENT

Of more than a hundred cases requiring insulin, this is the first one in which three doses of insulin a day, when matched with a properly proportioned diet, has failed to control the hyperglycemia and glycosuria.

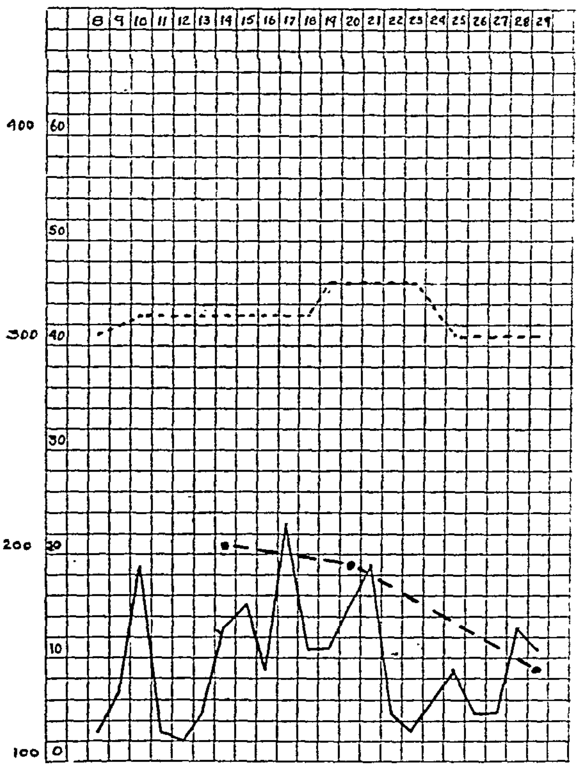

Chart 6.—Compare with Chart 1. Notice lower insulin dosage properly spaced through the day and night produces better results than a large dose three times a day, as in Chart 1.

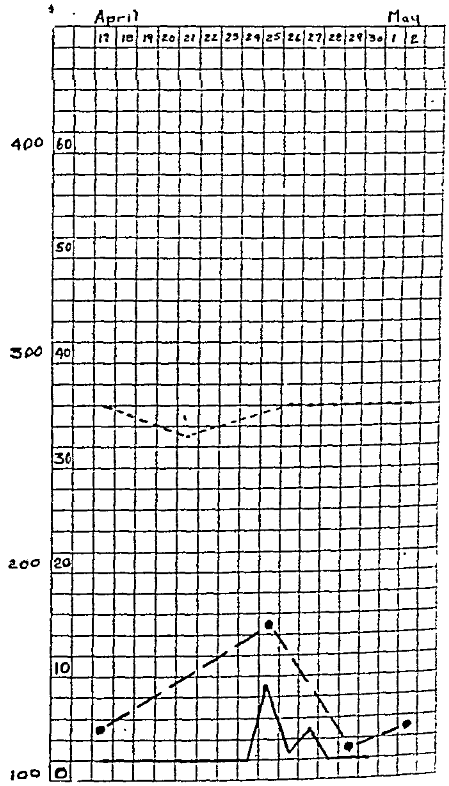

Chart 7.—Compare with Chart 1 and Chart 6.

This case demonstrates that a patient on a weighed diet, receiving insulin three times a day before meals, may show a high blood sugar in the morning, daily excretion of sugar, and still develop hypoglycemia of a dangerous degree.

The hypoglycemia is obviously due to the overlapping effect of insulin doses during the day. The hyperglycemia and glycosuria occurring between midnight and the morning dose of insulin may mislead one and lead to the dangerous increase of dosage of insulin.

CONCLUSION

In a patient apparently not responding to increasing doses of insulin by a proportionate decrease in sugar excretion and reduction of blood-sugar level, the time relation of sugar excretion to insulin dosage should be investigated. The simplest method is the examination of the three-hour portions of urine. Frequent blood-sugar determinations give additional information.

The remedy is the redistribution of insulin in amount and at the time when required.

NOTE.—Since the above was written, four additional cases have been observed which behaved in a similar manner, that is, they required the administration of insulin some time between *midnight and 6 A.M. in order to maintain the blood-sugar level approximately normal.*

BLOOD GAS ANALYZER*

BY C. C. GUTHRIE, M.D., PH.D., PITTSBURGH, PA.

THE importance of blood gas analysis entitles it to a place in undergraduate medical physiology and pharmacology. It is particularly instructive in conjunction with simultaneous measurement and analysis of expired air, in rebreathing experiments, and in pharmacologic studies. The available forms of such analyzers are too costly for some medical schools to purchase, or require more time for mastery by the average student than is available in such courses. For these reasons the following apparatus was devised. Its class use for three years has been so satisfactory that it seems desirable to describe the design and record data essential to its evaluation.

The apparatus is a modification of the Haldane form[1] without the control tube and supplied with a pressure equalizer consisting of a cylinder and hollow glass plunger in place of the conventional reservoir and flexible tube. So constructed, the apparatus is very compact and inexpensive (Fig. 1).

The burette (*A*) is made from a one c.c. pipette graduated in hundredths, by bending the suction end back upon itself just above the zero calibration mark and removing the constricted portion of the tip. The equalizing cylinder (*B*) is fitted with a hollow plunger (*C*) consisting of a piece of thick-walled glass tubing having an internal bore the same as that of the burette. The plunger should be fitted snugly but not tightly so that it may be easily raised and lowered. If necessary, the final fitting may be perfected by rubbing down the plunger with carborundum paper. The lower or curved end of the displacing cylinder is closely connected to the tip end of the burette by means of a short rubber sleeve (*D*). The assemblage is mounted on a wooden support (*E*) by means of two brass spring clips (*F* and *G*) attached with screws. A flat brass spring (*H*) is mounted just above the upper end of the equalizing cylinder and so adjusted that it presses moderately against the plunger to

*From the Department of Physiology and Pharmacology, School of Medicine University of Pittsburgh, Pa.

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insure that the plunger will remain stationary in all positions. The wooden base measures 11 by 2¼ by ½ inch. In the middle of the back, three inches from the top, a rod ¼ by 1½ inches, threaded for ½ inch is screwed into a hole provided for the purpose and serves as a support for clamping the apparatus to a stand (*I*). The wooden base is painted black and a strip of white glazed paper pasted to the front before mounting the burette to serve as a background for accurate reading. The dimensions of the glass parts are such

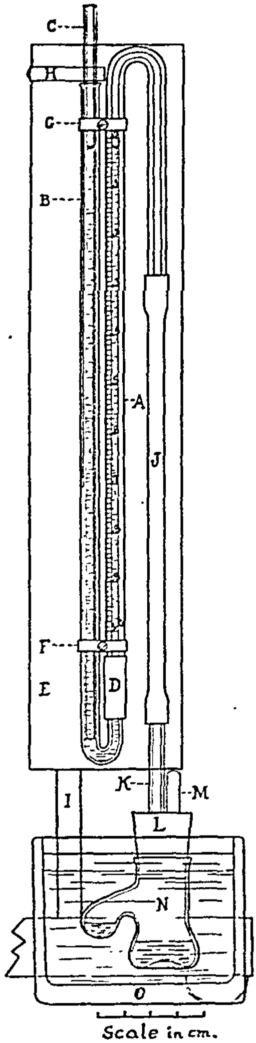

Fig. 1.

that when mounted the tubes do not project beyond the margins of the base, excepting the upper end of the plunger which should be of such length that when thrust to the bottom of the cylinder the upper end projects ½ inch above the base. The free end of the gas burette is connected by means of a seven-inch length of thick-walled pure gum tubing (*J*) to a straight glass tube (*K*) having a bore of 4 mm. The glass tube is inserted into a hole of a two-holed No. 4 rubber stopper (*L*) until the end is flush with the under surface. To close the other hole in the stopper a piece of glass rod (*M*) about one inch

long is employed. A glass vial (N) provided with a side pocket is connected with the stopper. The neck of the vial is of such size that a secure fit is obtained when the stopper is inserted ¼ of an inch. The total height of the flask is 1⅝ inches. The bottom is flattened and flared. The neck of the side pocket arises near the middle of the wall. The neck should not be less than ⅜ of an inch in diameter and the pocket is depressed and flared so that it holds at least 0.5 c.c. without spilling over into the vial when the vial is placed upright on the table. The total capacity of the vial with cork in position is 13 c.c. The base is clamped to the stand and an 8 oz. ointment jar (O) of water is set beneath and the apparatus lowered until the vial is suspended in the water so that the upper surface of the water comes to the level of the junction of the rubber stopper and vial mouth.

Alcohol, or water to which a little bile salts have been added,[2] is used for the displacing liquid. Before connecting the vial, or before sealing the vial with the glass plug, with the plunger pushed down as far as it will go the alcohol level should be at slightly above zero on the burette.

Accessory apparatus comprises a thermometer, pipettes calibrated for measuring 0.5 and 1 c.c. of blood and an aerating bottle, which will be described with the directions for its use.

The reagents are (1) ammonia solution. This consists of one part of strong ammonia (sp. gr. 0.88) made up to 250 parts with boiled distilled water.[3] To this a small quantity of saponin powder is added to accelerate laking of the red blood corpuscles and several drops of caprylic alcohol to prevent foaming on shaking after adding the blood. This is conveniently kept in a bottle provided with a pipette graduated to measure 1.5 c.c. (2) A saturated solution of potassium ferricyanide. (3) A 20 per cent solution of tartaric acid. Both the ferricyanide and tartaric acid solutions are kept in small bottles provided with dropping pipettes. (4) Saponin powder.

APPARATUS ASSEMBLAGE AND ADJUSTMENT

Push the displacing plunger well down. The column of alcohol in the tubes should read near, or slightly above zero. Remove the glass plug and connect the rubber stopper to the tube so that when it hangs down, the hole in the stopper for the glass plug is directed forward. Attach the vial to the stopper firmly. Place the water-bath in front of and against the stand and beneath the vial so that the vial hangs near the middle of the bath. By raising and lowering the apparatus on the stand, make adjustment so that the vial is submerged to the top of the lip. Note the room and bath temperatures. The temperature of the bath should not be more than about 0.5° C. below the temperature of the room. Do not work in a draught, in direct sunlight, or near a radiator.

CONTROL TEST

Place two drops of the ferricyanide solution in the vial pocket and 1.5 c.c. of the ammonia solution in the vial. Insert the stopper and place the vial in the water-bath, taking care not to mix the reagents. Agitate the water in the bath for five seconds by holding to the stopper and giving the vial a gentle

circular motion. Moisten one end of the glass plug and insert it about ¼ inch in the hole in the stopper. This will depress the alcohol in the gas burette. Equalize the levels of the two columns of alcohol by raising the displacing plunger. At about one minute intervals, stir the water-bath with the vial by grasping the stopper only, as previously described. As may be necessary, readjust the levels of the alcohol columns until they show no change for a period of one minute. This will indicate the zero reading. The burette may be read to 0.1 of a space, i.e., 0.001 c.c. In general, the zero reading should be adjusted to slightly below the zero mark. When necessary to readjust for this, it is easily accomplished by removing the glass plug and readjusting the alcohol levels to slightly above zero before reinserting the plug. After zero is established and recorded, by grasping the stopper, lift the vial from the bath and thoroughly mix the contents of the vial and pocket by tipping and gently shaking from side to side for two minutes. Do not permit any of the liquid to get on the stopper. Replace the vial in the bath and observe any change in the volume of gas by the technic above described for establishing zero level, excepting that the glass plug is not touched. When the volume has become stabilized, note any change. Any volume change is obtained by subtracting the zero from the final reading.

Remove the glass plug, unstopper the vial and wash, rinse with distilled water at room temperature and dry it.

OXYGEN CAPACITY OF BLOOD

Blood is prepared by placing about 5 c.c. in a clean, dry, six-ounce, wide-mouth bottle, replacing the stopper and rotating horizontally for five minutes. Shaking or agitating with sufficient violence to cause foaming is avoided.

The procedures described for the control test are now repeated and in addition, 1 c.c. of the oxygenated blood is placed under the ammonia solution. The surplus blood from the point of the pipette is removed with a pledget of cotton before putting it under the ammonia solution. Shake gently to mix the blood and ammonia solutions and wait at least five minutes for the zero reading in order *to insure that the blood will have become completely laked before mixing with the ferricyanide solution.* In mixing the ferricyanide with the blood and ammonia and shaking, avoid the formation of bubbles as they interfere with the accuracy of the results. As the gas is given off keep the alcohol levels approximately the same by raising the displacing plunger. The final reading should be obtained in from four to five minutes. The end-point, of course, is indicated by the volume becoming stable. Note the bath temperature. The final result is expressed in cubic centimeters of oxygen per 100 c.c. of blood. Reduce this to dry gas at 0° C. and 760 mm. Hg. pressure. Table 1 is adequate for student use.

OXYGEN CONTENT OF ARTERIAL BLOOD

This is determined as in the case of oxygen capacity, excepting that the blood is not exposed to air but removed from beneath oil and placed in the vial under the ammonia. (Caution.—On placing the blood under the ammonia solution do not agitate until the zero reading has been established.) Set zero at about 0.10, then shake gently to lake and continue until the blood is saturated

TABLE I

FACTORS FOR REDUCING TO DRY AIR AT 0° C. AND 760 MM. OF AIR SATURATED WITH MOISTURE AT DIFFERENT TEMPERATURES AND PRESSURES. PRESSURE 735 MM. HG.

15° C.	16°	17°	18°	19°	20°	21°	22°	23°	24°	25°
0.898	0.894	0.889	0.884	0.880	0.875	0.870	0.865	0.860	0.856	0.850

with oxygen. Read the burette to determine the amount of oxygen absorbed.* Then mix with the ferricyanide and determine the total amount of oxygen.

The second reading subtracted from the first reading gives the amount of oxygen absorbed in the vial; while the first reading from the last reading gives oxygen content of the blood when placed in the vial. The second reading from the last reading gives the oxygen capacity but the method first described is recommended.

OXYGEN CONTENT OF VENOUS BLOOD

The technic is the same as for the oxygen content of arterial blood. From the oxygen capacities of the arterial and venous blood samples the oxygen contents can also be expressed in percentages of saturation.

CARBON DIOXIDE CAPACITY OF BLOOD

Direct Method.—This is determined with the same apparatus as used for oxygen capacity and the general technic is the same. In addition to carbonate-free ammonia solution and saturated ferricyanide solution, 20 per cent tartaric acid solution is required. It is most important, if carbonate is present in the ammonia solution, that the quantity be known so that the figure for blood can be corrected. To determine this, place 4 drops of tartaric acid in the vial pocket, 1.5 c.c. of ammonia solution in the vial and proceed as described in the control experiment under oxygen capacity.

To determine the carbon dioxide capacity of blood, place 5 c.c. in a six-ounce, wide-mouth bottle provided with a tight stopper through which two glass tubes extend into the bottle. The tubes are closed by small stoppers or caps. After the blood has been placed in the bottle, add a little dry saponin powder and agitate occasionally until the blood is *completely* laked. This should occur in about five minutes. Then add two drops of saturated ferricyanide solution and shake gently until the oxyhemoglobin has been changed into methemoglobin. This requires not more than five minutes. It can be determined spectroscopically without removing the blood from the bottle. If oxyhemoglobin is present, on subsequent addition of tartaric acid and shaking, some of it will be liberated, thus interfering with the accurate determination of carbon dioxide.

The blood is now exposed to alveolar air until the carbon dioxide content becomes stabilized. To do this, unstopper the tubes and at the end of expiration forcibly expel all the air possible from the lungs into the bottle. Do this two or three times to insure that all of the air originally in the bottle has been displaced by expired air. Then quickly stopper the tubes, place the bottle horizontally on the table top and roll it to and fro so that the blood forms a film around the sides of the bottle. After two or three minutes, again

*For observing the rate of absorption as well as the total amount of oxygen absorbed, a method including a description of a constant shaking apparatus has been described by McEllroy and Guthrie, Am. Jour. Physiol., 1920, ii, 105.

expire into the bottle as before, close the tubes and continue to roll the bottle for several minutes. If the bottle temperature is below the dew-point, some moisture from the breath will condense. In such case, the expired air may be introduced through a bottle of glass beads or other device in order to remove the surplus moisture. Ordinarily, this is unnecessary.

Place four drops of tartaric acid in the pocket of the vial and 1.5 c.c. of the ammonia reagent in the vial and with the pipette place 0.5 c.c. of blood under the ammonia solution. In doing this, avoid exposure of the blood to room air. The vial is stoppered and a test for carbon dioxide carried out as before described.

The actual volume percentage of carbon dioxide is calculated from the burette reading as follows:

1. Correct the reading by subtracting the amount of carbon dioxide given off by the ammonia solution alone on mixing with the acid as determined in the control test.

2. Add 2.0 per cent of this volume to correct for dilution of blood by ferricyanide in aerating bottle and double this to express the amount of carbon dioxide in 1 c.c. of blood.

3. To correct the reading for the amount of carbon dioxide remaining dissolved in the solution, multiply the number of degrees the temperature of the bath is above 13° C. by 0.5, subtract this from 120 and multiply the corrected gas reading by the remainder.[4]

4. By reference to the table given under oxygen capacity, correct the gas volume for water vapor, temperature, and pressure.

CARBON DIOXIDE CAPACITY OF BLOOD

Indirect Method.—To determine the carbon dioxide capacity of blood by the indirect method proceed as in the direct method, placing five c.c. of blood in the aerating bottle, adding saponin powder to lake.

A. After laking the blood, saturate it as previously described by exposure to alveolar air. Remove 1 c.c. and determine the oxygen content as previously described.

B. Again expose the blood in the bottle to alveolar air and place 0.5 c.c. under the ammonia solution in the testing flask, into the pocket of which two drops of ferricyanide solution and four drops of tartaric acid solution have been placed and determine the total oxygen and carbon dioxide content.

The actual volume percentage of carbon dioxide is calculated from the burette reading as follows:

1. From the total volume, subtract the amount of carbon dioxide given off by the ammonia solution alone, on mixing the acid and one-half of the oxygen content of 1 c.c. of blood as determined in the previous tests.

2. Correct for the amount of carbon dioxide remaining dissolved in the solution as described under the direct method. A more exact correction would be the addition of 21.5 per cent less a fortieth for each degree above 13° C., but the difference in the final result would be of little significance.

3. Double this to express the amount of carbon dioxide in 1 c.c. of blood.

4. Correct the gas volume for water vapor, temperature, and pressure.

OXYGEN AND CARBON DIOXIDE CONTENT OF ARTERIAL AND VENOUS BLOOD

The blood is collected under oil and analyzed for oxygen alone, and for total oxygen and carbon dioxide as described under the indirect method for carbon dioxide capacity under A. and B., excepting that the blood, as in the case of the oxygen determination alone, is transferred from under the oil to under the ammonia solution.

By determining the oxygen capacity of the blood by saturating in alveolar air the oxygen contents of arterial and venous blood in terms of percentage saturation may be expressed.

DISTRIBUTION OF OXYGEN AND CARBON DIOXIDE IN BLOOD

Divide a sample of oxalated blood. Centrifugalize one sample to obtain plasma. Determine the oxygen and carbon dioxide capacity of each. By determination of the volumes of the sediment and of the plasma, the actual quantitative distribution in the whole blood may be calculated.

SUMMARY

The methods have yielded sufficiently accurate results for the purposes for which they have been employed. Check tests are always made, and in reasonably practiced hands, usually vary not more than 5 per cent. Results for oxygen are more uniform than for carbon dioxide. Oxygen capacity tests are the most accurate and uniform while carbon dioxide content tests are least uniform. As with all gas methods, increasing skill is shown by greater uniformity of results, and repeated tests have demonstrated that in only moderately trained hands, results by these methods certainly are as accurate and uniform as by other current methods, while the time necessary to learn the technic as well as the time required for making tests is shorter.

REFERENCES

[1]Haldane, J. S.: Respiration, Yale University Press, 1922, p. 410.
[2]Haldane, J. S.: loc. cit., p. 412.
[3]Haldane, J. S.: loc. cit., p. 416.
[4]Haldane, J. S.: loc. cit., p. 416.

BASIC FUCHSIN FOR ENDO MEDIA*

By R. W. French, Washington, D. C.

PRELIMINARY consideration of the action of the basic fuchsin-sodium sulphite combination in an Endo medium discloses the fact that the reaction is practically independent of changes in hydrogen-ion concentration, but in reality that the combination is acting largely as a Schiff's reagent, detecting aldehydes produced by bacterial metabolism. This statement may be readily proved experimentally, whereby it is found that sensible variations in P_H utterly fail to influence the appearance of color in such a medium.

*From the Laboratory Service, Walter Reed Medical Center, Washington, D. C.
Received for publication, June 5, 1925.

With this fact established the following test for the suitability of a basic fuchsin for use in Endo media is suggested:

In a test tube take 10 c.c. of a 0.05 of 1 per cent aqueous basic fuchsin. Determine the amount of 0.5 of 1 per cent aqueous sodium sulphite necessary to effect decolorization. Then determine the amount of 0.5 of 1 per cent formalin (40 per cent formaldehyde) necessary to restore a distinct fuchsin color (the original color will not be restored).

In use, this test first will show that samples of fuchsin vary considerably in performance, some requiring very little of the sulphite to effect complete decolorization, likewise little of the aldehyde to restore the color. Other samples will require many times the amount of sulphite and formaldehyde for appreciable results.

The application of this test on the different types of basic fuchsin reveals some interesting facts. First, in the case of a new fuchsin or a para-rosaniline hydrochloride a very appreciable precipitate forms immediately on the addition of the sodium sulphite, while with rosaniline hydrochloride no precipitate is noticeable for some time. Second, the addition of but a few drops of the formaldehyde solution restores the color to the decolorized rosaniline hydrochloride, while many times the amount of formalin is necessary to effect a partial restoration of color to the new fuchsin. Third, the precipitate formed on the addition of the sodium sulphite persists even after adding the formalin. Filtration further shows that the filtrate from the para-rosaniline hydrochloride is colorless (that the formalin has failed to restore any trace of color) while the filtrate from the new fuchsin shows but a very slight restoration of the original color.

These results, which parallel quite well those obtained in the investigations by the Biological Stain Commission,* show that rosaniline hydrochloride is thoroughly satisfactory for the preparation of an Endo medium, that new fuchsin is partially so, and that para-rosaniline hydrochloride is entirely unsatisfactory.

From the degree of the reaction in the test tube it is apparent that a small portion of the new fuchsin molecule acts similarly with sodium sulphite to rosaniline hydrochloride, which is not surprising when it is considered that chemically the two compounds are similar, the former having two more methyl groups in meta position to the methane carbon. It also seems, judging from the great difference exhibited by para-rosaniline hydrochloride, that the methyl group in meta position to the methane carbon in rosaniline hydrochloride is an essential to the developing of a color by the aldehyde after decolorization of a fuchsin by sodium sulphite.

Quantitatively, rosaniline hydrochloride is much the more satisfactory for Endo media than any other of the basic fuchsins, or mixtures thereof. The color is developed quicker and to a much greater degree than is the case with samples of new fuchsin ordinarily, and where this latter dye works satisfactorily or on a parity with the rosaniline, it is undoubtedly due to an admixture of the latter dye.

NOTE: Since the preparation of this article reference by Muller, compt. rend. soc. biol., 1924, xciv, 653-655, is noted in which it is shown that an acid also is necessary to accomplish the reaction. Any organic acid, such as lactic acid, may be used, and as a rule there is sufficient formic acid in the formaldehyde to bring about the reaction.

*Science, lx, No. 1556, pp. 387-388.

LABORATORY METHODS

A NEW HEMOGLOBIN SCALE*

By C. E. Roderick, M.D., Battle Creek, Mich.

THE small instrument shown in Fig. 1 is used for the estimation of hemoglobin. It is especially designed for the use of the practicing physician at the bedside.

It is not claimed that this instrument will replace the Dare, Fleischl-Meischer and others of the more expensive type which are to be found in the Clinical Laboratory.

It consists of a color scale graduated from 10 to 100 per cent. One hundred per cent being equivalent in color to a standard base on 13.77 gm. of hemoglobin in 100 c.c. of serum. This is the standard used by other instruments of present-day use.

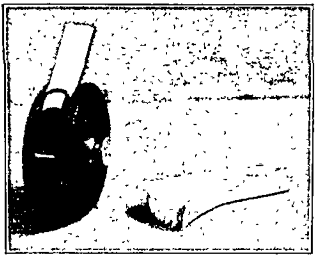

Fig. 1.

While no paper color scale can be relied upon to determine low percentages of hemoglobin with complete accuracy, this scale agreed with the Dare instrument in a large number of cases where the hemoglobin was found to be forty per cent or more, and when there was disagreement, it was seldom more than eight per cent.

The technic for use of this instrument is simple:

A small leaf of paper is removed from the pad (Fig. 2-A) and a drop of blood is soaked up. This is then placed in the small frame above the aperture and the edge brought into apposition with the color scale inside (Fig. 3). The scale is then turned by means of the little thumb-screw and the color is matched and reading made directly at the base of aperture.

*From Battle Creek Sanitarium.

Read before the Fourth Annual Convention of the American Society of Clinical Pathologists at Philadelphia, May 20-23, 1925.

The advantages of the instrument are:

1. Small and compact; easily carried in the vest pocket, Fig. 4.
2. Simple to operate.
3. Economical; uses small pieces of paper which are made up in booklets.
4. Efficient in that the areas of blood and scale compared are about 1 cm. square.

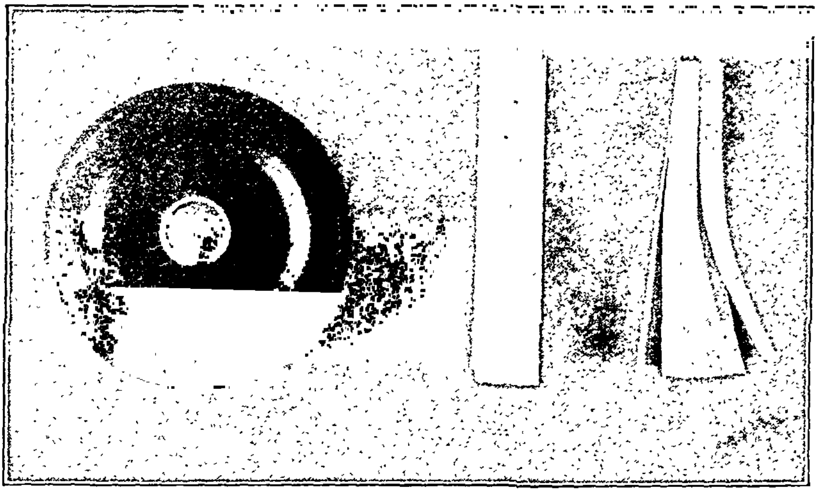

Fig. 2. A.

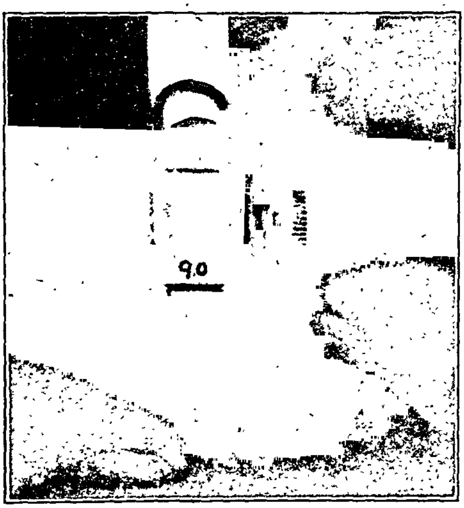

Fig. 3.

Fig. 4.

5. Scale is covered, with exception of one shade, when not in use, thus fading is prevented.
6. Made of metal and will wear for years. Additional booklets of paper may be had, thus eliminating the necessity of purchasing a new scale each time the paper supply is exhausted.
7. Should paper scale fade or become otherwise damaged it may be replaced without the necessity of securing another instrument.

ANILIN AS A DECOLORIZING AGENT IN THE GRAM STAIN*

By Hardy A. Kemp and Moyer S. Fleisher, St. Louis, Mo.

A SCORE or more of modifications of the Gram technic for staining and differentiating bacteria have been offered since Gram's original work in 1884. Most of these modifications have for their purpose the elimination of those variable factors which render the results obtained with this method more or less uncertain in the hands of the inexperienced, and even at times in the hands of more experienced workers. Using the original Gram technic, the principal cause of variations in results seemed to center on the decolorizing agent, namely, alcohol.

In an effort to find a method which would give constant results, even when used by the beginner in bacteriologic technic, we were led to try various modifications in which a decolorizing agent other than alcohol was used. We used successively acetone, acetone-alcohol, acetone-phenol, acetone-ether, phenol, and alcohol in percentages ranging from twenty-five to absolute. In these, as in all later experiments except when expressly stated, Staphylococcus aureus was used as the representative Gram-positive organism, and Friedländer's bacillus as the Gram-negative organism. The results with all of these reagents were, however, indifferent at best, and apparently uncontrollable factors, or factors very difficult to control, entered into the differentiating by means of all of these agents. Even when a rigid technic was used, they did not give constant results with the two test organisms.

There is mention made in Mallory and Wright's *Pathological Technique*,[1] of Weigert's method of staining bacteria in tissues. Weigert's technic[2] is essentially similar to the Gram method for staining bacterial smears, excepting that anilin is used as a decolorizer and no counterstain is employed. We have found no other references in the literature concerning the use of anilin as the decolorizing agent in the Gram stain. The results which we have obtained with anilin as a decolorizing agent have, however, been strikingly constant.

We found on decolorizing with anilin after staining according to Gram's method, using however ammonium oxalate gentian-violet[3] in the first step, that the counterstain in every instance brought out the Gram-negative bacillus in unmistakable contrast to the Gram-positive coccus. In all cases in which the smear had been thoroughly washed in the anilin, no bacillus could be found which retained the violet color, nor were there any red staining cocci. Subsequent trials with other organisms gave similar results.

The technic employed is as follows:

1. Ammonium oxalate gentian-violet applied to the smear for one minute.
2. Wash off excess stain in tap water.

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Fig. 1 is an oblique view of the tool, and Fig. 2 gives the detail of the ring. The ring must now be wrapped with thread in order to obtain even distribution of the paraffin. Cotton thread will operate as a wick and No. 20 has been found a satisfactory size to use. Fig. 3 is a detail, several times enlarged, which illustrates the method of wrapping.

In use the tool is dipped into ordinary paraffin heated to the point where it begins to smoke. It is then placed lightly but securely on a dry slide, withdrawn quickly, and the paraffin allowed to cool. Three rings can be placed on an ordinary three inch slide (Fig. 4), and by practice rapidity of technic is easily developed.

Rings of 16 mm. diameter will hold two large drops easily and allow for manipulation of the slide to effect mixing. Stirring with a flat wooden toothpick, however, will produce a much more intimate intermingling of the drops. Of course, a separate toothpick is used for each ring and then discarded. After a ten to twenty-minute incubation the whole slide may be tilted and manipulated to produce enough agitation to break up any pseudoagglutination. Cover slips cannot be used with paraffin rings. In order to prevent evaporation of the specimens during the incubation period, it is necessary to place them in a covered humidor. At the above-named hospitals the humidors used are ordinary staining dishes with wet blotting paper on the bottoms. These are illustrated in Fig. 5.

If it is necessary or desirable to use the slides again, they may be cleaned satisfactorily by boiling vigorously in a washing soda or soap powder solution and then allowing them to cool. The soap or soda dissolves dried sera or other substances, while the heat and boiling action dislodge the paraffin. When cold, the scum of solid paraffin can be removed from the top and the slides rinsed in clear water.

A NOTE UPON THE DESTRUCTION BY HEATING OF COMPLEMENT IN HUMAN SERUM*

By Robert A. Kilduffe, A.M., M.D., Atlantic City, N. J.

As a result of numerous and extended studies of the various factors influencing the application of the complement-fixation test to the serologic study of syphilis, in a majority of methods in common use, the test is conducted upon sera inactivated by heating.

The primary purpose of this inactivation is the destruction of the natural complement and of thermolabile anticomplementary substances.

Various workers have demonstrated, however, the thermolability of syphilitic reagin, and, to minimize this destruction, the period of inactivation has been reduced. Kolmer[1] recommends fifteen minutes as sufficient, and this period is rather widely used.

Davis,[2] in view of the fact that there may still be occasional doubt as to

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the efficacy of this shorter period, reported the complete destruction of complement in five hundred sera inactivated for fifteen minutes at 56° C.

The present note is in further corroboration of the efficiency of this period at this temperature.

During a study of the relation of the natural hemolysins of human sera to Kolmer's quantitative complement-fixation test for syphilis, the results of which have been reported elsewhere,[3, 4] two hundred sera were subjected to a temperature of 56° C. for varying periods up to fifteen minutes and then tested for the presence of natural complement.

All of the sera tested contained in 0.1 c.c. sufficient natural antisheep amboceptor to completely hemolyze 0.5 c.c. of 2 per cent sheep cell suspension.

The sera were placed in test tubes in a water-bath at 56° C. and 0.1 c.c. removed after five, seven, ten, and fifteen minutes, placed in fresh tubes, and 0.5 c.c. of 2 per cent sheep cell suspension added and the mixture incubated in a water-bath at 38° C. for one hour.

The presence or absence of complement was determined by the occurrence or absence of hemolysis.

The results of the series were as follows:

After inactivation at 56° C. for five minutes hemolysis occurred in eight sera, or 4 per cent.

After inactivation at 56° C. for seven minutes no hemolysis occurred in any of the sera, and the same was true of the sera tested after ten and fifteen minutes' exposure to this temperature.

Exposure to a temperature of 56° C. for fifteen minutes is, therefore, an efficient procedure for the destruction of the natural complement of human sera.

REFERENCES

[1]Kolmer, J. A.: Infection, Immunity, and Biologic Therapy, 1924, ed. 3, 446, W. B. Saunders and Co., Phila., Pa.

[2]Davis, G. E.: A Note upon the Destruction of Complement in Human Sera by Heating at 56° C. for Fifteen Minutes, Jour. Lab. and Clin. Med., March, 1925, ix, 6, 475.

[3]Kilduffe, R. A.: The Relation of the Natural Antisheep Hemoysins of Human Sera to Kolmer's Quantitative Complement-Fixation Test in Syphilis, Arch. Dermat. and Syph., December, 1924, x, 734.

[4]Kilduffe, R. A.: The Influence of the Natural Hemolysins of Human Sera upon the Production of Anomalous Reactions in the First Tube of Kolmer's Quantitative Complement-Fixation Test for Syphilis, Jour. Lab. and Clin. Med., November, 1924, x, 2, 93.

and soda lime in this removes all gas from the effluent air. The large volume of air passing through the line necessitates a series of equalizing bottles M, M', M'' to quiet the fluctuations in the pull of the pump N.

Pump N not only draws the gas-air mixture through chamber H, but at the same time pulls air through the dog box (b, Fig. 2) via entrance O and exit P. The amount is controlled by the by-pass Q.

A size B. Leimann air pump V, capacity 250 liters per minute, is used to draw gas-air samples from the chamber H during the period of exposure through the tube or bubbler S filled with a suitable absorbent and inserted in the gassing chamber H at R. The rate of flow through the tube S is registered by the flow-meter T and controlled by the by-pass U. The usual rate of flow used is ten liters per minute.

Consult Fig. 2, dimensions are given in feet and inches. Entrance to dog box b is made by removing lid c. Lid c is fastened down by means of clamps on all sides, such as d, shown in figure. To insure an air-tight connection between b and c a rubber gasket is used. Dog cage e is supported and moved by carrier g from dog box to gassing chamber and vice versa. The front end

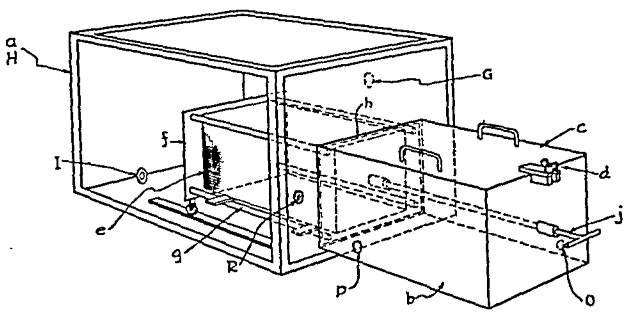

Fig. 2.

of carrier g is fastened to rod j with a swivel connection, and the movements of carrier g are controlled by the manipulation, by hand, of this rod. When the carrier is in the dog box, an air-tight fit is made between rear-end of carrier and rear-side of connection h by means of a tongue and groove circuit, and a nut-screw lock on rod j. To lock, turn j several times to the right. It is necessary that this connection be air-tight to prevent a leakage of gas from gassing chamber into dog box prior to the exposure. When carrier g is in the gassing chamber, the front end of the carrier fits plumb against the front side of connection h, and is held there by pressure of hand upon rod j.

The procedure for exposing a dog is as follows: The dog is placed in the dog box and the dog box closed and sealed tight. Air pump N is started and a flow of 200 liters per minute maintained through the gassing chamber. The heating baths E and E' are turned on. Sample tube or bubbler S is fastened to flow-meter T, and pump V run for a short time to get the desired flow through tube S. As soon as the substance X has been heated to the desired temperature, the nitrogen is turned on and the gas-air mixture drawn through the chamber to build up the desired concentration. At the end of this period, rod j is unlocked by a certain number of turns to the left, and the dog is quickly pushed into the gassing chamber. At the same time pump V is started, and

tube S inserted into the gassing chamber at R. During the exposure pressure is exerted upon the rod j by the operator; the flow-meters and heating baths are watched and regulated if necessary.

At the end of the exposure these operations take place simultaneously: the dog is quickly withdrawn from the gassing chamber and rod j given several turns to the right; tube S disconnected from the gassing chamber and pump V stopped; by-pass Q is closed. When by-pass Q is closed the rate of flow of air through the dog box increases from fifty to over one hundred and fifty liters per minute, which is rapid enough to completely clear it of gas. The sample tube or bubbler S is disconnected and its contents analyzed. The analytical result divided by the flow through S will give the concentration in milligrams per liter. The dog box is then opened and the dog removed for further examination and observation.

We wish to express our acknowledgment to Lieut. Colonel E. B. Vedder (M. C.), U. S. A., for valuable suggestions and to Mr. Harry Shannon for practical help in building this chamber.

Ohio Society of Clinical Laboratory Diagnosis

The Sixth Annual Meeting of the Ohio Society of Clinical Laboratory Diagnosis will be held in Toledo, Ohio, on the 14th and the 15th of May, 1926, following the meeting of the Ohio State Medical Association. Dr. Jonathan Forman, President, and Dr. C. M. Hyland, Secretary-Treasurer.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

Balachowsky, S.: Hemotonia: A New Factor Determining the So-called Sedimentation Velocity of the Erythrocytes. Ann. de Med., September, 1925, xviii, 201.

The author reports his studies of the Fahraeus phenomenon as a result of which he believes that the mechanism of the reaction involves the existence of a previously undescribed factor which he denominates as "hemotonia."

The method used for the test was as follows:

Into a capillary pipette with a diameter of 1 mm., which has first been washed with 5 per cent potassium oxalate, a large drop of blood is drawn from a finger puncture. The column of blood must be from 4 to 10 cm. in height. The pipette is then kept in a vertical position and a reading made, at thirty minutes, of the height of the plasma column above the red cells, from which reading the average hourly velocity of sedimentation can be calculated.

For the detailed study of the mechanism of the reaction the author devised the following technic:

A small receptacle was made by attaching four cover-slips with wax to a slide. The receptacle thus manufactured measured 0.1 to 1 mm. in depth, 10 mm. in height, and 0.5 mm. in width. He suggests that a Burker cell can be used for this purpose.

The receptacle was placed on the stage of a microscope, inclined at an angle of 90°, and filled with oxalated blood.

The reaction was then observed with a micrometric eyepiece.

With the low power there is seen between the mass of red cells and the supernatant plasma, a very distinct and apparently rectilinear line of demarcation. Under the high power, however, this line is found to be irregular, notched, and suggestive of the outlines of a geographical map.

The supernatant plasma shows the presence of rouleaux and conglomerate masses which, after accidental detachment from the general mass of red cells, remain adherent to the walls of the glass cell or seem to be repelled by a current.

These isolated elements fall very rapidly as soon as the masses are freed, accidentally or artificially. Their fall is much more rapid than that of the general mass of erythrocytes.

On examining the general mass of red cells there are no free erythrocytes, all being united in rouleaux which, in turn, often form conglomerate masses. These masses and the rouleaux form a network the spaces of which are filled with plasma. This network has three dimensions and is termed, by the author, the "skeleton of the blood."

The detailed observations described are summarized as follows:

1. The red cells forming rouleaux and conglomerates in the blood constitute a network which is the skeleton of the blood.

2. Under the influence of weight and through a series of local depressions there occurs a general heaping of the blood skeleton on the bottom of the receptacle.

3. This blood skeleton is not deprived of solidarity for: (a) The configuration of a given portion may remain unchanged for a long period of time. (b) The free upper border of the bulk of the erythrocytes preserves its shape for a long period. (c) There exists a force of resistance against the collapse of the blood skeleton other than the viscosity.

4. The solidity of the blood skeleton, being a function of the tonus of its constituents, is the new factor denominated by the author as "hemotonia."

The author believes that these experiments and observations demonstrate that the sedimentation of erythrocytes is not, as has been assumed, merely a simple phenomenon of sedi-

mentation, but a more complicated reaction in which the effects of heaping play an important part. For this reason he would denominate the phenomenon as the "sedi-compilation of erythrocytes."

Rosenow, E. C.: The Precipitin Reaction in the Diagnosis of Scarlet Fever and Allied Hemolytic Streptococcus Infections. Jour. Am. Med. Assn., Jan. 2, 1926, lxxxvi, 9.

Rosenow reports a precipitin test for the diagnosis of scarlet fever and other conditions due to hemolytic streptococci.

The immune scarlatinal serum was prepared by the intravenous injection of a horse with freshly isolated hemolytic streptococci obtained from scarlatinal sore throats.

Small tubes are made from thin-walled glass tubing having an inside diameter of 3 to 3.5 mm. cut into lengths of 3.5 cm. and sealed at one end.

A capillary pipette made from the same tubing with a capillary outside diameter of about 0.5 mm. is used to add the extract and serum.

Material for extraction is secured by swabbing the nasopharynx, contact with the tongue being scrupulously avoided.

The swabs are made of absorbent cotton wrapped around the roughened end of aluminum wire (1.5 mm. diameter). The swab is then bent at an angle of 60°. The extraction may be made immediately or after the swab has dried while kept in a sterile tube.

The swab is placed in 2 c.c. of sterile normal saline or in 0.2 per cent gelatine Locke solution and thoroughly agitated. The cotton, removed from the end of the wire with forceps, is expressed by wrapping it around the end of the forceps while pressing it against the wall of the tube.

The resultant mixture is cleared by centrifugation at high speed in narrow tubes. The clear fluid is taken up with the capillary pipette, the end of which is then placed at the bottom of the precipitin tube and enough of the fluid ejected to make a column about 1 cm. in height. The pipette is then rinsed in three changes of normal saline and an equal volume of immune serum added, the tip of the pipette being again placed at the bottom of the tube so that the serum lies beneath the extract.

Extract and serum should be water clear or, at most, only slightly opalescent

The tubes are incubated two hours and then read and a second reading is made after standing in the ice chest overnight. The two readings usually agree.

Readings are best made in a darkened room by transmitted light.

Negative: absence of cloud at zone of contact.

Plus-one: slight cloud.

Plus-two: moderately heavy cloud.

Plus-three: heavy, slightly granular cloud.

Plus-four: heavy cloud with precipitation.

Robin, A.: A New Method for Enriching the Stools. Compt. rend. Soc. de Biol., Paris, May 1, 1925, xlii, 1099.

1. Introduce a piece of feces about the size of a nut into a flask containing about 20 glass beads and 14 c.c. of Schweitzer's reagent (formula not stated). Mix thoroughly and rapidly.

2. Pour off the liquid obtained into two centrifuge tubes and centrifuge for one minute.

3. Pour off the supernatant fluid and wash the outside of the tubes, held vertically, by rapid agitation in water without disturbing the sediment. Add 4 c.c. of normal saline and agitate thoroughly.

4. Filter through a metallic sieve with a mesh of 340 microns.

5. Add 4.5 c.c. of a mixture of HCl 22 parts, formol 34 parts, and normal saline 44 parts. Shake for fifteen seconds. Add 12.5 c.c. of ether and shake vigorously for about fifteen seconds.

6. Divide the mixture into two centrifuge tubes and centrifuge one minute.

7. Examine the sediment. If cysts are not seen, it is necessary to remove with a pipette a little of the mixture at the junction of the ether with the fluid.

Ova and cysts are but very slightly changed in form and structure.

Nadler, W. H., Starr, P., and Tukey, G.: Glycemia as a Guide to the Treatment of Diabetes Mellitus. Arch. Int. Med., October, 1925, xxxvi, 579.

It is generally recognized that repeated blood-sugar estimations are an invaluable if not, indeed, an essential feature of the treatment of diabetes. The development of micro methods for the determination and of means for the preservation of the blood samples has greatly facilitated such studies but the necessity for repeated visits to the laboratory by fasting patients is still a source of annoyance.

The authors, after a study of various micro methods and methods for the preservation of the sample, have devised a procedure whereby blood can be collected by the patient, preserved, and forwarded to the laboratory as convenient.

For the collection of the sample small, wide-mouthed vials (7 × 12 mm.), fitted with a rubber stopper were utilized. In each bottle is placed 5 mg. of a 10:2 finely powdered mixture of sodium fluoride and thymol.

Patients were supplied with an automatic spring lancet, the point of which had been sharpened to resemble a Hagedorn needle, and taught how to prick the finger and milk approximately 0.2 c.c. of blood into the vial. The blood and the chemicals were then mixed with a toothpick, the vial stoppered, and sent to the laboratory by mail.

About 8 drops of blood was sufficient.

Samples so collected showed no marked loss in blood sugar, for as long as eleven days, when examined by a micro method (that described by Randles and Griggs, Jour. Am. Med. Assn., Mar. 1, 1924, lxxxii, 684).

Soldin, M., and Lesser, F.: Study of Induced Serum in Syphilitic Infants. Deutsch. med. Wchnschr., Oct. 30, 1925, li, 1817.

The authors report the consistent demonstration by dark-field illumination of Spirocheta pallida in the serum from blisters induced by cantharidin-collodion on the skin of syphilitic infants in whom syphilitic eruptions were present.

The method was not successful in adults.

Boyers, L. M., Kofoid, C. A., and Swezy, O.: Chronic Human Amebiasis. Jour. Am. Med. Assn., Nov. 7, 1925, lxxxv 1441

The authors, in a well illustrated paper, report a review of the diagnosis and treatment of chronic human amebiasis on the basis of encystment in the liver area.

They recall that it is a common inference that encystment of E. dysenteriae occurs only in the colon and call attention to the fact that it is logical to believe that an early, mild hepatitis is a concomitant or sequel of the mild colitis occurring as a characteristic sign of amebiasis in the majority of cases.

Microscopic studies, which are illustrated in the paper, bring abundant evidence of entrance of amebae into the blood stream from whence they may pass to the liver through the hepatic portal stream. The hematogenic distribution of amebae from colonic infection is thus provided for and, in the opinion of the authors, occurs more frequently than has hitherto been recognized.

This they believe to be responsible for the existence of chronic amebiasis as a clinical entity.

The necessity for a careful and minute history is emphasized, salient symptoms being fatigability and "bowel consciousness."

They emphasize that the condition may be mistaken for a variety of diseases.

To detect hepatic colonization by amebae they resort to duodenal drainage according to the method outlined below.

The patient is examined without breakfast. The ordinary Rehfuss duodenal tube is passed and the patient placed on a couch on his right side. A group of four flasks is made ready. Into flasks 2 and 4 is placed approximately 50 c.c. of Bouin's solution. (Saturated aqueous solution picric acid 75 parts; glacial acetic acid 5 parts; formalin 20 parts.)

The end of the tube is placed in flask 1 and allowed to remain there until the fluid begins to siphon away. When the fluid in flask 1 begins to show a tinge of bile, the tube is

passed into flask 2 and allowed to drain for about one hour. Then 125 c.c. of warm saturated solution of magnesium sulphate is introduced with a glass syringe and as much of the solution as possible is withdrawn by the syringe. The tube is then allowed to drain into flask 3 until all the magnesium sulphate is passed and dark cystic bile begins to flow, when the tube is allowed to drain into flask 4.

The so-called hepatic bile may, if desired, be collected in a fifth flask containing the same fixative solution.

The material in flasks 2 and 4 is then centrifuged and the sediment, the cellular part of which is already fixed, stained directly or the sediment may be infiltrated in paraffin, sectioned, and stained and then examined.

By this method encysted amebae may often be demonstrated.

Sturtevant, M.: Achlorhydria Preceding Pernicious Anemia. Jour. Am. Med. Assn., Nov. 21, 1925, lxxxv, 1638.

The absence of HCl in the gastric contents is a common finding in pernicious anemia and the possibility of its causal relation to this condition has been considered.

Sturtevant reports a case of known achlorhydria of fourteen years' duration prior to the onset of the symptoms of pernicious anemia.

The symptoms of achylia began in 1899; the diagnosis of achlorhydria was made in 1900 and the symptoms of pernicious anemia detected in 1916.

He believes the case to be the longest as yet recorded.

Lederer, M.: A Form of Hemolytic Anemia Probably of Infectious Origin. Am. Jour. Med. Sc., October, 1925, clxx, No. 4, 500.

Lederer reports the observation of three cases characterized by sudden onset with elevation of temperature, leucocytosis, and a severe anemia resembling pernicious anemia, all of which symptoms promptly and rapidly disappeared following a single transfusion.

He comments upon the paucity of similar clinical observations and believes the condition not infrequent but, perhaps, obscured by unfamiliar terminology.

The outstanding features are the acuteness of the symptoms, evidence of very rapid erythrocytic destruction, extreme regenerative action of the bone marrow, rapid recovery after a single transfusion, and the absence of sequelae.

In all of the cases the stained smear showed a very close resemblance to the picture seen in pernicious anemia during a crisis. Because of the large number of erythroblasts, the leucocyte count was corrected by subtracting from the number of white cells estimated by the white cell pipette, the number of erythroblasts estimated from the stained smear.

The leucocyte counts varied from 25,800 to 52,000 with 51 to 85 per cent polymorphonuclears. Enormous numbers of erythroblasts were found, 3000 per c.mm. in one instance.

Red cell fragility was normal in all cases and all the usual laboratory examinations were negative.

The spleen and liver were enlarged in two cases and the eyegrounds in two cases showed recent changes of a type commonly found in pernicious anemia. Bone marrow tenderness was absent.

The leucocyte count is tentatively explained as possibly due to three factors: infection, increased production due to the stimulation of a toxin, and heaping up. The nature of the infection or the character of the toxin remain unknown.

Bott, C.: The Diagnosis of Cancer in the Blood Serum. Münch. med. Wchnschr., Nov. 13, 1925, lxxii, 1959.

Bott reports his experience with the serologic test for cancer devised by Wigand.

Blood is secured by venipuncture, chilled, and the serum removed. The test should be made promptly after securing the specimen.

A series of dilutions of the serum with normal saline is then set up in twenty-four tubes, the dilutions increasing in "geometrical progression." The final volume in each tube is 2 c.c.

To each tube is then added 1 c.c. of a freshly prepared and filtered 1 per cent

solution of tannic acid and two or three drops of a "strongly diluted" solution of carbolfuchsin, the purpose of which is to render flocculi more visible.

Readings are made of the degree and extent of flocculation at twelve, eighteen, and twenty-four hours.

In normal cases flocculation occurs to Tube 12 at twelve hours, to Tube 16 at eighteen hours and to about Tube 18 at twenty-four hours. The reaction is negative if there is no flocculation beyond these points. Carcinoma "of slight extent" gives a reaction similar to that seen in normal cases; advanced carcinoma, and also late pregnancy, show flocculation to the limit of the serum dilutions.

Bott considers the reaction a lability reaction, nonspecific, and of dubious value, disagreeing with Wigand in these respects.

Patten, A. C.: The Human Cerebrospinal Fluid in System and Diffuse Degenerative Disease Involving the Nervous System. Amer. Jour. Med. Sc., Jan., 1926, clxxi, No. 1, 48.

The author tabulates, from the literature, a large number of group studies made in a variety of conditions.

The findings in some of the more frequent conditions are thus summarized:

Multiple Sclerosis:

Pressure: no significant variations.

Protein: increased in 50 per cent of cases; no uniformity.

Cell count: 0 to 42 per cu. mm.; no pleocytosis in 75 per cent of cases.

Colloidal gold: inconstant, ranging from negative to luetic reactions.

Sugar: no uniformity.

Creatinin: 1.78 mg. per 100 c.c.

Urea: 15.5 mg. per 100 c.c.

The Encephalitides:

Pressure: variable, generally increased.

Protein: increased (72 per cent of cases).

Cell count: may vary from normal to several hundred; mononuclears predominate; greatest number of cells in the first three weeks, then declines.

Sugar: ranges from 0.062 to 0.095 mg. per 100 c.c.

Results of examination are constant only in inconsistency.

Poliomyelitis:

Pressure: normal or slightly increased.

Protein: slight to moderate increase.

Cells: early high count: lymphocytes and large mononuclears predominate; later variable to normal.

Wassermann: negative.

Colloidal gold: luetic zone reaction.

Sugar: increased.

Epilepsy:

Pressure: normal.

Cells: normal.

Globulin: negative to slight increase.

Wassermann: negative.

Colloidal gold: negative.

In the encephalitides the Wassermann is negative and the colloidal gold reaction inconstant, varying from luetic to paretic curves.

Numerous other neurogenous, traumatic, infectious, and degenerative conditions are reported, the results being very inconstant.

Plass, E. D., and Mathers, C. W.: Placental Transmission III: The Amino Acids, Non-protein Nitrogen, Urea, and Uric Acid in Fetal and Maternal Whole Blood, Plasma, and Corpuscles. Bull. Johns Hopkins Hosp., June, 1925, xxxvi, No. 6, p. 393.

In view of the discordant findings reported in the literature, this question was restudied by the authors. The methods of Folin and Wu were used throughout on specimens obtained from the maternal arm vein and the umbilical cord.

The amino acids are uniformly higher in normal fetal whole blood and plasma than in the maternal blood and plasma.

The total nonprotein nitrogen is always higher in the fetal blood. Urea is usually the same in both bloods.

Uric acid is generally the same; if there is a difference, it is generally higher in the fetal blood.

The article contains numerous tables.

Isaacs, R.: Effect of Roentgen Ray Irradiation on Red Blood Cell Production in Cancer and Leucemia. Amer. Jour. Med. Sc., Jan., 1926, clxxi, No. 1, p. 20.

It is generally accepted that small doses of x-rays have a stimulating and large doses a depressing effect upon living tissues, the latter effect being due to tissue death.

Isaacs endeavored to study the mechanism of the depressant action by determining the variations in the red cell count after therapeutic irradiation in cancer and leucemia. Attention was focussed particularly upon "granule red cells," mature corpuscles containing a single refractive granule 0.5 micron in diameter and representing an intermediate stage between the reticulocyte and the normocyte, and the reticulocytes.

In health these corpuscles vary from 1 to 3 per cent. An increase above three per cent usually means abnormal blood production. Variations above three per cent are of more significance than decreases below this figure. The cells are only demonstrable by vital staining.

Blood was examined before and after irradiation in a series of 30 patients (carcinoma, 17; leucemias, 10; erythremia, 2; and chronic hemolytic jaundice, 1).

Blood films were stained supravitally with cresyl blue, using Wright's stain as a counterstain.

Differential counts were made of 1,000 to 5,000 red blood cells.

The degree, rate, duration, and site of radiation varied in accordance with the case.

In susceptible cases irradiation stimulated changes which are normal in the life of a red blood cell, only the rate and time of initiation being varied.

Following effective short-ray radiation, in one to twenty-four hours one of several responses may be noted. The granule cells may show an increase, appreciable or negligible, temporary or sustained.

Where the increase is relative or absolute there may be a relative or absolute decrease in reticulocytes unless the marrow is hyperplastic (hemorrhage).

Where the increase of granule cells is significant and sustained (two or more days), definite clinical improvement is seen in cancer and leucemia, the reverse being also true.

Where the increase is temporary and followed by a leucopenia, there has been too much radiation and the patient may become worse clinically.

The most frequent effect of radiation is an increase, gradual and temporary, in the number of red cells per cu. mm. and an increase in the color index which may rise above one.

The change in the percentage number of immature red blood cells is one of orderly stimulation to reach maturity.

The percentage changes of immature cells appears to be a systemic effect of localized exposure and the changes in the relative percentages are similar to those following transfusion.

The period between the application of irradiation and the beginning of maturation changes is probably a matter of minutes as compared to hours for the leucocytes and weeks for tissue cells.

From the evidence of the effect upon young red blood cells, all roentgen ray irradiation may be considered as stimulative: small doses stimulate to division; large doses may stimulate to maturity and death by senility, the rate for each tissue varying with its specific growth properties.

Brown, G. T.: Bacterial Vaccines in Asthma. Amer. Jour. Med. Sc., Jan., 1926, clxxi, No. 1, p. 94.

There has been so much discussion regarding the use of bacterial vaccines in asthma that Brown details a method of diagnosis and treatment which has given very good results in his hands.

A careful and minute history and physical examination are essential preliminaries upon which he justly lays great emphasis.

These are followed, *when indicated,* by such special examinations as x-ray, when they seem advisable and then by skin tests with such pollens, proteins, and bacterial proteins as may be placed under suspicion by the history. He does not believe in the indiscriminate testing of everybody with everything and calls attention to the importance of looking for delayed reactions.

When the leucocytes are over 10,000 and the polymorphonuclear count above 80, a careful search is made for a focal infection. Lymphocytosis calls for the elimination of tuberculosis, lues, or a latent streptococcic infection. A Wassermann is made in all cases of continuous asthma as many of these will clear up under antiluetic treatment.

He does not agree with the importance given to asthma as a cause of eosinophilia and, when this is present, advises a careful search for other causes.

When all investigations have failed, he assumes that the case has been shown, by elimination, to be due to bacteria. Cultures are then made of the sputum, organisms isolated in pure culture, and a vaccine prepared from each, containing two billion organisms per cubic centimeter. Intradermal skin tests are then made with each vaccine, injecting enough to produce a blister ¼ inch in diameter and using as a control normal saline containing a trace of agar as this is generally present in the vaccine.

Only those vaccines are used for treatment to which the patient reacts. With this method very favorable results have been obtained.

Kubie, L. S., and Schultz, M. G.: Vital and Supravital Studies of the Cerebrospinal Fluid and of the Meninges in Cats. Bull. Johns Hopkins Hosp., August, 1925, xxxvii, No. 2, p. 91.

The investigation reported was concerned with a study of the varied cell pictures seen in the cerebrospinal fluid and commonly associated with imflammatory processes, with particular reference to "pus cells," "macrophages," and "fibroblasts"; their origin, and significance and how far they may be capable of transformation from one form to another.

The difficulty in the cytological study of stained smears of the spinal fluid in inflammatory processes has been that the slow evaporation of the large amount of watery fluid in which the cells are suspended has produced a marked shrinkage and distortion.

In these studies the essential data were secured from a study of the living cells. A new method was also devised for the fixation of cells without much distortion, which is described below.

A strip of glass is sealed to the bottom of a Petri dish with a little balsam. A few crystals of iodine are scattered loosely over the bottom of the dish, which is warmed gently just before using, until faint purple fumes fill the chamber.

A coverslip, containing a single large drop of spinal fluid, is then placed on the strip of glass in the dish.

The cells are fixed almost at once. The drop itself dries quite slowly but when it is dry the cells have settled down upon a small circular area of the slip, firmly attached to the glass surface.

The preparation is now deeply stained with minute crystals of iodine and is overlaid with a fine film of coagulated protein.

The excess iodine of this preparation is washed out with a strong aqueous solution of potassium iodide, after which it is rinsed in water and stained. The authors used Mayer's carmalum to secure a contrast with trypan-blue inclusions, which substance they used to produce the inflammatory reactions, and for a more detailed study of the cell morphology, the Unna-Pappenheim combination of pyronin and methyl-green.

For the study of living cells the method described by Sabin, was utilized (Bull. Johns Hopkins Hosp., Oct., 1921, xxxii, 368; ibid. Sept., 1923, xxxiv).

The paper is very detailed in its discussion of the observations made and illustrated and should be read in the original.

The results are thus summarized:

In the first twenty-four to forty-eight hours after the onset of meningeal irritation,

polymorphonuclear leucocytes predominated, being later usually outnumbered by mononuclear forms.

In relatively chronic inflammations lymphocytes predominate and appear to accumulate in the perivascular channels, being partly released by drainage of the spinal fluid and more competely washed out by the simultaneous intravenous infusion of hypotonic solutions. This procedure may have possible therapeutic applications.

It is suggested that the so-called plasma cell is identical with the intermediate lymphocyte. Phagocytes arise from young phagocytic forms normally present throughout the meninges.

The cerebrospinal fluid accelerates the degeneration and inhibits the motility of the polymorphonuclear neutrophilic leucocytes and the lymphocytes to a marked degree. It is less destructive to the eosinophilic leucocyte and the phagocyte.

Lipshutz, B., and Lowenburg, H.: Pneumococcic and Streptococcic Peritonitis: Report of Twenty-three Cases in Infancy and Childhood. Jour. Am. Med. Assn., Jan. 9, 1926, lxxxvi, 99.

Commenting upon the high mortality, practically 100 per cent, of this condition, the lack of efficient means for its treatment, and the fact that it is not as infrequent as has been supposed, constituting about 10 per cent of the acute abdominal emergencies of childhood, Lipshutz and Lowenburg suggest the necessity for its serious study.

They report twenty-three cases occurring within three years.

The disease appears to be idiopathic. The streptococcus was usually of the hemolytic variety. Typing of the pneumococcus was not done. None of the pneumococcic cases showed any pulmonary lesion, as demonstrable by physical examination or radiography.

They consider it very significant, however, that a history of mild upper respiratory or pharyngeal infection was present in every case in which the peritonitis was of pneumococcic origin.

The authors believe that there is a distinct relation between coryza or throat infections and pneumococcic or streptococcic peritonitis.

They review the four possible portals of entry:

1. The blood stream. In nine cases a positive blood culture was secured.

2. The lymphatic tract, which seems improbable and conflicts with the pathology of the condition.

3. By the genital tract. Pneumococcic peritonitis is most common in girls (3:1). The series reported shows that, with one exception, all the pneumococcic infections were in girls.

The pneumococcus was isolated from the vaginal secretions, peritoneum, and blood in three cases. The peritonitis usually begins as a pelvic peritonitis. The disease is most frequently encountered where there are unhygienic conditions. For these reasons the authors believe that the genital tract may be an important source of infection in this disease.

4. The gastrointestinal tract also fits in with the clinical and pathologic studies of this disease and gastrointestinal disturbance was present in every case. It is difficult, however, to produce peritonitis experimentally by using the gastrointestinal tract as the experimental portal of entry.

The paper discusses in detail the relation of appendicitis in infancy to pneumococcic and streptococcic peritonitis.

Peritoneal puncture and examination of the exudate by smear and culture is advocated as a diagnostic measure. Untoward results from this procedure were not encountered.

The clinical picture is described in detail.

A serious study of this infection by the pediatrician, interne, surgeon, and laboratory worker is urged.

Birkhaug, K.: Demonstration of Antigenic Relationships Among Strains of Streptococcus Erysipelatis by Intradermal Protection Tests. Bull. Johns Hopkins Hosp., Aug., 1925, xxvii, 2 to 85.

This paper is one of a series of studies on the biology of Streptococcus erysipelatis and reports a method whereby these organisms may be differentiated from the group of streptococci associated with scarlet fever and numerous other streptococcic infections.

The study reports the results of intradermic tests conducted on rabbits.

An area 8 x 12 cm. was depilated on both sides of the chest and abdomen and then divided into smaller areas approximately 4 cm. in diameter. These small areas were then infiltrated with 0.1 c.c. of immune sera (erysipelas serum prepared by the immunization of rabbits, scarlatinal antistreptococcic serum, and heterologous antistreptococcic serum). After an interval of twenty-four hours to allow fixation of the immune bodies, into the center of each area thus passively immunized 0.1 c.c. of a hemolytic streptococcus emulsion from a 24-hour blood agar slant suspended in 10 c.c. of Locke's solution was injected intradermally.

The pathogenic activity of Streptococcus erysipelatis was inhibited only by the erysipelas serum. Nonspecific protection was not afforded by any of the other sera nor by normal rabbit serum.

By this method of local passive immunity it was possible to differentiate a group of hemolytic streptococci causing erysipelas from a group of hemolytic streptococci causing scarlet fever on the one hand, and from the large series of miscellaneous hemolytic streptococci producing a variety of pyogenic infections on the other.

Rienhoff, Jr., W. F.: Histologic Changes Brought about in Cases of Exophthalmic Goiter by the Administration of Iodine. Bull. Johns Hopkins Hosp., November, 1925, xxxvii, No. 5, p. 285.

The administration of iodine in the treatment of exophthalmic goiter produces both gross and microscopic changes in the gland, which have been studied and are reported by Rienhoff in an excellently illustrated article.

The tissues were Zenker-formalin fixed and stained with hematoxylin-eosin.

The histologic changes produced by iodine are thus summarized:

(a) An increase in the size of the thyroid gland as a whole.

(b) A decrease in the vascularity and probably in the lymph flow throughout the gland.

(c) A large increase in the amount of fibrous connective tissue.

(d) A striking increase in the amount of colloid deposited and contained within the thyroid gland.

(e) A change in the acini from lace-like papillomatous ingrowths to round, even-walled, smooth acini, regular in size and form.

(f) A transition of epithelium from high columnar to flat cuboidal and occasionally low columnar.

(g) A change in the nuclei of epithelial cells from large clear nuclei to the small, irregular, pyknotic type.

(h) The presence of many mitotic figures before the use of iodine and their absence after iodine has been given.

The histologic picture following the use of iodine appears to be constant.

Mikulowski, W.: Diagnostic Value of Bacilli of Koch in the Feces of Children. Arch. de med. des Enf., April, 1925, xxviii, 201.

In view of the fact that it is difficult to secure specimens of sputum from children, by whom sputum is frequently swallowed, the author examines the feces for tubercle bacilli, using the technic described by Besancon and Jong.

To about 30 grams of feces in a sterile glass sufficient 25 per cent solution of sodium cholride is added to make a semiliquid mass by trituration. The mixture is then filtered into two centrifuge tubes through sterile gauze, 2 c.c. of a mixture of equal parts of ether

and ligroin added to each tube, the tubes stoppered, thoroughly shaken, and centrifuged for ten minutes at 5000 r.p.m. Immediately below the ether layer is a cake about 2 mm. thick in which the tubercle bacilli, if present, will be found. Smears are made from this cake and stained by the usual methods.

Frei, W.: A New Skin Reaction in Inguinal Lymphogranuloma. Klin. Wchnschr., Nov. 5, 1925, iv, No. 2, p. 48.

In the course of a study of five cases of this condition the author devised the skin test detailed below.

From a soft focus in a bubo, pus was aspirated and diluted 1:10 with normal saline and the mixture heated for two hours at 60° C. and again, on the following day, for one hour at the same temperature. It was then cultured for sterility.

Into two patients and three normal controls, 0.1 c.c. of the diluted sterile suspension of pus was injected intracutaneously in the deltoid region.

In twenty-four hours only a slight reddening at the site of the injection was observed in the controls. In the lymphogranuloma cases, however, there was an inflammatory reaction 0.75 to 1 cm. in diameter associated with the formation of papules. The reaction increased in intensity in thirty-six to forty-eight hours, when the inflamed area measured 2 to 3 cm. in diameter, and then gradually subsided with slight superficial necrosis.

Repetition of the test gave similar results varying somewhat in intensity in accordance with the source of the antigen.

Complement-fixing bodies were not detected.

There was no difference between the controls and the cases when injected with pus similarly prepared and obtained from staphylococcal infections, tuberculous glands, or material from tuberculous glands, doubtful malignant granuloma, syphilitic liver extract, or Ducrey bacillus vaccine.

The antigen from lymphogranuloma cases gave no reaction in patients with other types of gland infections, such as: syphilis, tuberculosis, malignant granuloma, lymphatic leucemia, or lupus.

It is suggested that the reaction is due to the presence in the pus of either the etiologic agent of the disease or its elimination or disintegration products.

If the reaction proves constant in a large number of cases, the author suggests that it may be of aid in differentiating lymphogranuloma inguinal from other localized glandular infections.

Wohl, M. G.: Hemophilia. Neb. State Med. Jour., Nov., 1925, x, No. 11, p. 442.

Wohl, in a case report, recalls the efficiency of a method of treatment which he first heard suggested by Da Costa.

A full grown rabbit is anesthetized; the liver is removed under aseptic precautions and ground in a sterile meat grinder.

About 3 c.c. of sterile normal saline is added for each gram of liver substance and the mixture allowed to stand for forty-five minutes to permit extraction of thrombokinase. The mixture is then filtered. Cotton saturated with the filtrate is applied as a dressing.

Sabin, F. R., Cunningham, R. S., Doan, C. A., and Kindwall, J. A.: The Normal Rhythm of the White Blood Cells. Bull. Johns Hopkins Hosp., July, 1925, xxxvii, No. 1, p. 14.

The sudy reported concerns itself with the mechanism involved in the remarkable constancy maintained in the number of leucocytes in the circulating blood.

Through the use of a supravital staining technic the number of white cells, their death and replacement, was found to vary in a definite rhythm. The staining method has been published by Sabin (Bull. Johns Hopkins Hosp., 1923, xxxiv, 277).

The article abounds in tabular statistics and in a detailed discussion of the various forms of white blood cells seen in the circulating blood. The results of the study may thus be summarized:

1. There is a characteristic rhythm of the total white blood cell count with an interval of approximately one hour's duration.

2. The total number of white blood cells varies in the proportion of 1:2.

3. There is an afternoon increase in the total white cell count, irrespective of the ingestion of food and due entirely to an increase in the number of polymorphonuclear neutrophiles.

4. The polymorphonuclear neutrophiles die out in showers, often of considerable proportions, the dead cells being promptly replaced from some reservoir as yet undetermined.

5. The lymphocytes have a shorter rhythm than the leucocytes, but the total number varies as 1:3 in contrast to 1:2 for the leucocytes.

6. A small proportion of monocytes are normally undergoing division in the circulating blood.

7. In five of six apparently normal individuals, small percentages of myelocytes were found in the circulating blood.

The paper contains a wealth of detail which does not lend itself readily to abstracting and should be read in its entirety by those interested in the subject.

Faupel, M. H.: A Comparison of the Kahn and Wassermann Tests. Bull. Johns Hopkins Hosp., Sept., 1925, xxxvii, 3 to 170.

A series of comparative tests on 400 patients is reported.

The Kahn test was performed by the procedure in vogue and described in 1923 by Fox and Sanderson (Amer. Jour. Syph., 1923, vii, 687); the Wassermann test was performed with a cholesterinized heart extract and cold incubation, no other details being given.

In general the coefficient of agreement was 0.79, corresponding to 90 per cent absolute agreement.

The results with spinal fluid were not nearly so favorable as those reported by Kahn.

The Kahn test is regarded as a valuable corroborative check on the Wassermann; but is not considered as replacing the complement-fixation test in the serologic diagnosis of syphilis.

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Official Organ of the American Society of Clinical Pathologists

EDITORIALS

The Argentaffine Tumors of the Appendix. What Are the So-Called Carcinoid Tumors?

PRIMARY epithelial tumors of the appendix have been reported with increasing frequency during the past few years. Considerable doubt has been raised as to the character of many of the early cases which were reported. In 1897 Letulle and Weinberg, and Mossé and Daunic reported in rapid succession primary epithelial appendiceal growths which did not correspond in clinical course to carcinoma, and showed no tendency to recur after removal of the appendix.

Statistics indicate that primary tumors of the appendix are rare. Williams, in studying 15,481 tumors, among which were 7,878 carcinomas, found no tumors of the appendix. Kelynack has stated that primary tumors of the appendix are practically unknown. In more recent statistics dealing with diseases of the appendix one cannot help being struck with the rather constant percentage with which the so-called primary carcinoma of the appendix is

found in a large series of appendices removed during the first or recurring attacks of appendicitis. Kelly reports in his series of 706 cases, two (or 0.3 per cent) cases of so-called primary carcinoma; A. and E. Moschcowitz, six (or 0.3 per cent) in 2,000; and McCarthy and McGrath, 22 (or 0.4 per cent) in 5,000. These rather constant percentages in different series of large numbers of cases would indicate that they were dealing with a lesion different from carcinoma. The rather constant percentage would suggest that this lesion might be found in any series in which the appendix was removed because of an inflammatory process or as an incidental finding in appendices removed during the course of other abdominal operations.

These growths are rarely multiple, but similar growths in the small intestine not infrequently are. The shortness of the appendix may account for the rare multiplicity. In a number of cases the appendix, the seat of such a growth, removed during an operation for some other abdominal lesion has been practically normal in appearance. In most of the cases, however, the appendix has been the seat of an acute or chronic inflammatory process. Frequently it is more or less adherent; at times the appendix is adherent only at the site of the tumor.

The form of the appendix depends upon whether there is kinking or torsion. The tumor is most frequently situated in the tip or distal third of the appendix. The appendix the seat of such a growth has a bulbous appearance, the bulbous enlargement often appearing nodular. In many of the cases that have been observed the epithelial growth has been found in a scar, or in a dilated portion distal to an obliterated section of the appendix.

The tumor may be so small that it escapes the naked-eye examination of the pathologist or surgeon, and a microscopic examination gives the first indication of the presence of such a growth. In most cases, however, the tumor is suspected because of the bulbous enlargement of the tip of the appendix, or a peculiar retraction or induration. Upon section the growth has a shiny, frequently transparent appearance. It is of firm consistency. The color is striking. While in many cases it is a grayish white, in the majority it is yellow, brownish, or even orange yellow in color. At times the growth appears caseous, suggesting a tuberculous lesion. At times the mucous membrane covering the growth appears yellow in color. This yellow appearance is due to the rich lipoid content of the cells and is of considerable diagnostic significance.

The submucosa is the principal seat of these growths. They are not, however, always sharply delimited. The changes in the mucosa vary considerably. It is usually thinner than normal, the glands are decreased in number, and degenerative changes are noted in the cells. The mucous membrane may be reduced in thickness, the cylindrical cells being flattened. Ulceration may occur in the late stages. When the growth is circular the lumen of the appendix may be obliterated. This may occur when the growth is confined to a portion of the wall of the appendix. As a rule there is no similarity between the tumor cells and those of the mucous membrane. Mossé and Daunic believe however that they have found a direct relationship, and Rolleston and Jones believe that genetically they are related. Hart has reported a case in which the glands

of the mucosa present a peculiar atypical growth, in parts resembling a carcinoma without infiltrating characteristics. The principal part of these growths always lies in the submucosa. The stroma varies in amount and kind in different cases and in different parts of the same tumor, transitions from a loose cellular to thick connective tissue with few cells being common.

Oberndorfer believes that an independent muscular stroma is peculiar to these growths, and Saltykow regards the muscle tissue as specific.

Forbus, in a recent contribution on this subject, states that the total number of cases of appendiceal carcinomata found in the literature up to 1923 was 317, and to this number have been added several recent cases. It would seem, therefore, of little importance to place on record further cases, all of which are characteristic and similar in every respect to those already described. And so, were it not for the fact that the true pathologic character of the primary tumors of the appendix and small intestine has been of late the subject of such widespread interest, and their clinical and pathologic characteristics the subject of such varying interpretation, a report of further cases would hardly seem justifiable.

One finds in the literature the following opinions expressed concerning the nature of these growths:

1. The tumor is a carcinoma, originating in the epithelium of the appendix.
2. That they are analogous to the basal cell tumors occurring in the skin.
3. That they develop from pancreatic rests or represent adenomyomas.
4. That they develop from the chromaffine cells of the crypts of Lieberkühn and are endocrine tumors developing from the paraganglionic system.

The following histologic description given by Forbus gives so well the character of the growth that it is given in toto, for the diagnosis can be made upon the grosser characteristics of the cells and their arrangement without resorting to the finer methods.

"The tumor tissue is composed of strands and small masses of round or oval cells embedded in an eosin-staining hyaline stroma. Almost all of the nests and strands of cells lie in the supporting tissue of the tumor but none of them contain tumor cells. The more one studies these spaces filled with tumor cells, the more one is impressed with their lymphatic-like appearance. In places flattened spindle cells exactly like endothelium are seen lining the spaces, although no place can be found where the spindle cells form a complete sheath about the tumor mass. No blood cells are found in contact with the tumor cells. Sometimes the cells distribute themselves about the space in such a way as to form an alveolar-like structure with a rather atypical and irregular lumen. This appearance seems, however, to be only an artefact. A true gland-like arrangement is quite definitely not present. In the more solid nests of cells no alveolar arrangement whatever is seen. The cells of the tumors are not confined entirely to the nodular mass in the appendix; a few strands and cell nests can be seen in the circular layer of muscle. The tumor is, therefore, not encapsulated but has very invasive and infiltrative characteristics. The cells which compose the tumor are round and oval in shape. The nuclei are round, comparatively large, quite regular in shape, uniform in size, and rich in chromatin which is distributed diffusely, though coarse granules may be

found in almost all of the nuclei. Nucleoli are quite frequently seen. The cytoplasm is abundant in some cells, but in the greater part of the tumor it is only moderate in amount in comparison with the size of the nucleus. Coarse granules, which have a rather reddish brown color, are found in most of the cells. Cytoplasm that contains no granules, on the other hand is full of vacuoles. Nowhere can cells be found which have a clear-cut columnar form, like the cells of the crypts of Lieberkühn.

"The stroma which forms the greater part of the tumor nodule consists largely of hyaline connective tissue. Small blood vessels are fairly common, but there are a few larger ones. Nerve fibers are numerous and present an interesting relationship to the tumor cells. In places the cells form an interrupted sheath about the nerve fibers. Toward the periphery of the nodule can be seen small groups of smooth muscle cells which are continuous with the circular coat of muscle surrounding the tumor. No muscle can be found with certainty within the tumor mass, although there are numerous isolated groups of spindle cells which morphologically are like smooth muscle. These do not, however, react characteristically to the differential stains. Nowhere can there be found any supporting stroma within the cell nests and strands. The cells are simply packed loosely together without any intervening tissue. Isolated groups of tumor cells and sometimes single cells can be seen far out in the muscle coat and in what appears to be the serosa. No metastases are found in any of the internal organs."

These histologic findings are quoted because Forbus believes that it is quite easy to differentiate between these growths and adenocarcinoma on a morphologic basis without depending upon the complicated and rather time-consuming silver impregnation method.

The cells of these tumors react to silver impregnation, which indicates their origin from chromaffine cells of the intestinal tract. These cells are found in the crypts of Lieberkühn throughout the gastrointestinal tract. They are situated between the cylindrical cells of the mucosa, some extending from basement membrane to lumen, while others remain without contact with the lumen. Silver staining granules are always demonstrable in these cells and are always located between the basement membrane and the nucleus. The vacuoles in the cells are probably secondary to the formation of granules.

Careful histologic studies with the proper technic have demonstrated the origin of these tumors from the cells of Koltschitzky and Schmitt in the crypts of Lieberkühn. They are tumors of the chromaffine system, the granules of the cells having the ability to reduce an ammoniacal solution of silver.

The clinical peculiarities and the rather constant percentage of occurrence in the different series of appendices removed at operation are explained by this new conception of the origin of these tumors. The term, argentaffine tumor, should be adopted for this group of tumors, as they are not malignant, even in those cases in which the cells have infiltrated to the serosa or into the fat of the mesenteriolum. A careful histologic study has led to the recognition of the character of this peculiar group of growths and has explained their rather constant percentage of occurrence and peculiarities in clinical course notwithstanding that in many cases they have decidedly invasive characteristics.

—*D. L.*

A Resume of the Scarlet Fever Situation

THE recent work of the Dicks has naturally been followed by a renewed interest in and generalized study of scarlet fever, its etiology, treatment, and prophylaxis.

Park,* in a recent paper, ably reviews the past and present work concerning this disease and outlines its present status in relation to the newer developments.

Because of its comprehensive character and the information it contains, the communication is here summarized.

Among the earliest to attempt to produce a curative serum was Moser, in 1902, who immunized horses with streptococci obtained from toxic cases of scarlet fever.

Savchemko, in 1905, demonstrated that the broth in which such cultures were grown contained a strong toxin and that, therefore, it should also be utilized in the preparation of curative serum. He demonstrated, too, the presence of antitoxic as well as streptococcidal bodies in the serum thus produced.

Gabritschewsky, in 1907, advocated the use of a vaccine containing both streptococci and the broth in which they were grown, and used such a vaccine in human beings with varying success in prophylaxis.

Encouraging reports were made of this product in 1921 by Poletevkova.

The work of the Dicks proved, for the first time, the etiologic relation of streptococci to scarlet fever and led to the development of their skin test for susceptibility and to the evolution of methods for the effective active prophylactic immunization of exposed individuals.

Through the work of the Dicks and Dochez, a scarlet fever antitoxin has also been produced, the results of the use of which are reported by Park.

After intravenous administration, very striking symptomatic results have been observed, and its early use very probably prevents the development of complications. After the rash has disappeared it is useless and has no effect on the later septic complications.

The effect on mortality has not as yet been striking, but it may be expected to become evident as the use of the serum extends and, especially, when its administration is early and in sufficient amount.

The adoption of a refined serum and a unit dose is strongly advocated. One unit is suggested as the quantity of antitoxin required to neutralize one hundred skin test (Dick) doses. The addition of a serum containing streptococcidal, as well as antitoxic, bodies, as advocated by Moser, merits careful investigation and is worthy of trial, as it is very likely to increase curative efficiency. —*R. A. K.*

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*Park, W. H.: Scarlet Fever: Etiology, Prevention by Immunization, and Antitoxic Treatment, Jour. Am. Med. Assn., Oct. 17, 1925, lxxxv, 1180.

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The Annual Meeting

The 1926 Convention of the American Society of Clinical Pathologists will be held in Dallas, Texas, April 15, 16, and 17, 1926.

Elaborate preparations are being made by our Texas members to give their colleagues a truly southern hospitable reception. Entertainment will be provided for the visitors and their wives. Drs. Moursund and Black have already called a conference of all the clinical pathologists of Texas at which plans were laid and arrangements perfected to make this meeting a memorable one in the annals of our organization.

Make Your Hotel Reservations Now!

In view of the great influx of physicians in Dallas for the American Medical Association meeting, hotel accommodations will necessarily be at a premium. Inasmuch as the A. S. C. P. meets during the week preceding the A. M. A. convention, there will be room for all our members during our own meeting, but unless reservations are made right now, the rooms cannot be held for those who desire to stay over the ensuing week.

The Baker Hotel has been reserved as headquarters for the A. S. C. P. This hotel offers ideal meeting rooms for our purpose.

All Clinical Pathologists Are Invited

It has become a tradition with the A. S. C. P. to invite all clinical pathologists to our annual conventions be they members or not. To this end about five hundred invitations have been sent out to nonmembers extending to them the facilities of the Society.

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CLINICAL AND EXPERIMENTAL

STUDIES ON THE BACTERIOLOGY OF THE URINE IN COOPERATION WITH CATHETERIZATION OF THE URETERS*

BY ROBT. A. KEILTY, M.D., DANVILLE, PA.

THE purpose of this paper is a report of the laboratory studies of urine from cases examined by cystoscopy and ureteral catheterization. The report includes microscopic and bacteriologic results as well as a clinical survey of the cases from the diagnostic standpoint.

The work is based on 162 cases, of which 50 have been reviewed in detail and tabulated in this paper. Frank cases of bladder, ureteral, and kidney involvement make up the majority (35) of the report but there are a large number (15) in which the examinations were made as a part of the routine study. In this latter number are patients giving vague abdominal symptoms which might be referable to most anything in which at least a careful urinary study with negative findings would be valuable from the standpoint of diagnostic elimination.

While I would not advocate the routine use of the cystoscope and ureteral catheter as one might a vaginal examination or the proctoscope, I am sure that its more frequent use where the urine is studied bacteriologically would clear up an otherwise obscure diagnosis, would eliminate the kidney or bladder as offenders, or would accurately clinch a diagnosis strongly suggested clinically.

For purposes of study the cases may be arbitrarily divided into three groups. First, those with clear-cut symptoms in which the laboratory merely confirms as, for example, the findings of streptococcus in a ureteral urine

*From the Department of Laboratories and Research Medicine of the Geo. F. Geisinger Memorial Hospital, Danville, Pa.
Read before the American Society of Clinical Pathologists, Philadelphia, Pa., May 21, 1925.

where the x-ray has already shown a stone. Second, those in which there are symptoms, for example, pus in the urine, where the laboratory makes a diagnosis of B. coli cystitis. Third, those in which there are vague symptoms and the laboratory findings complete the negative side of the clinical examination, thus eliminating the urinary system. In this study of 50 cases, 21 are in Group I, 14 in Group II and 15 in Group III.

Complete cooperation with the clinical service is necessary so that observations may be accurately recorded and different phases compared. Specimens must be sent to the laboratory with the least possible chance of contamination, clearly and correctly labelled. It has been my pleasure to work with J. P. Shearer and E. H. Adams, most of the cases with the latter and I am indebted to them for the best kind of cooperation.

The methods employed have been as simple as possible with a routine which has varied very slightly since the work was first undertaken. The patients receive a preliminary routine urine examination and often the presence of pus cells is the first indicator of a serious trouble. In this routine the presence of albumin, casts, pus cells, white blood cells and blood cells with types of epithelium are considered the most important elements to be watched for. Amounts indicated by 1, 2, 3, 4, are recorded for positive findings. While other things such as crystals, debris, urates, and bacteria are set down they are considered of secondary importance.

When the patient is put on the list for a cystoscopic examination, special specimens of urine are obtained under conditions as nearly aseptic as is surgically possible. About 10 c.c. in a test tube is sufficient except where tubercle bacilli are suspected when it is better to collect five or six test tubes from each ureter.

The specimens are given a number and are examined,—bladder, right ureter, and left ureter in order. The amount, color, sediment, precipitate, gross blood, and pus are noted. The specimens are centrifuged at high speed and the sediment used for all further examinations. Fresh smears are examined under dry powers with reference to white blood cells, pus cells, red blood cells, types of epithelium, and bacteria. Gram stained slides are next examined with the oil immersion for the presence and types of bacteria. Smears are fixed on slides on a copper plate, stained for tubercle bacilli by the cold method, which I advocated several years ago, and examined for at least five minutes and much longer where tuberculosis is suspected or where the tubercle bacillus has been found previously in a twenty-four-hour specimen. Cultures are made in a gas tube of glucose broth and by smear on blood agar plates. The cultures may be made the first thing before centrifuging or by loop transfer afterwards. Gas tubes, when positive, are transplanted to Endo's medium for B. coli and when there is a cloud and no gas, they are transplanted to blood agar plates. It is rarely necessary to fish colonies to separate plates since almost every case shows but one infecting organism. There is no flora in the bladder under perfectly normal conditions and the urine is probably sterile most of the time.

This technic is applied to each of the three specimens in order. Where an organism out of the ordinary is found, special media and methods are

used to work out special characteristics. In this series which I realize is very small, I have been dealing with a surprisingly small number of different species. Where a vaccine is in order, salt solution suspensions of scraped blood agar plates are made from the original cultures, if pure and heavy enough, or if not, from the first transplants available.

There are 162 cases in the series at this time and 50 have been carefully reviewed.

These include cystitis, 6; cystitis with pyeloureteritis and urethritis, 8; pyeloureteritis, 9; pyeloureteritis with stricture or pregnancy or renal calculi, 7; hydronephrosis, 1; pyonephrosis, 2; nephrolithiasis, 3; tuberculosis of the kidney, 3; tumor of the kidney, 1; and ptosis of kidney, 1. The negative cases include neurasthenia and neurosis (observation negative), chronic appendicitis, myocarditis and gall bladder, perineal tear and pelvic symptoms, gonorrheal prostatitis, purpura, chronic salpingitis, tuberculous epididymitis and Bartholin's abscess.

There were 12 males and 38 females. These types of cases are by far more frequent in the females. The youngest was five, the oldest sixty-four, average thirty-four years; the largest number were between the ages of twenty-five and thirty and again between forty and fifty, thus these diseases belong to middle adult life. The longest duration of symptoms was five years, the shortest one week, the average fifty weeks, and the greatest number between one and six months; the acute cases were between two weeks and two months and the chronic cases between one and two years.

In a study of the symptoms produced there is a wide variety, but pain of some character and position occurred in every case. Frequency was present 36 times, burning during and at the end of micturition 21 times, hematuria 18 times, nocturia 13 times. Then follows about equally distributed a long list of symptoms which were noted, such as backache, headache, weakness, chills and fever, tenderness, dribbling, etc. Involvement of some part of the urinary tract is characterized by pain, frequency, burning, hematuria and nocturia in order of frequency in this series.

Nineteen cases showed frequent and intermittent attacks before coming to the hospital, emphasizing the passage in cycles of these forms of urinary diseases.

In the preliminary urine report albumin was present in forty-three specimens from a trace to 4, mostly the former. This was due to blood or pus and not to an organic nephritis. Blood cells show up 21 times and pus 28, an exudative reaction proving the infectious nature of many cases in this class. Tubercle bacilli were demonstrable in twenty-four-hour specimens 3 times and were not found twice where specifically looked for.

In the cystoscopic specimens taken from the bladder fresh smear examination, pus cells appeared 26 times, white blood cells, 3; epithelium, 15; red blood cells, 18; bacteria, 6. Gram stain,—bacteria, 19; tubercle bacilli were never found in these specimens. Cultures,—tubes sterile, 9; gas positive, 20; cloud and no gas, 10; Endo positive, 12; blood agar plates, sterile, 17, growth, 23. B. coli occurred 17 times, Streptococcus hemolyticus, 6; Staphylococcus

albus, 5, hemolytic staphylococcus, 1; B. fecalis alcaligenes, 1; Streptococcus pyogenes, 1; undetermined B. coli Group II.

In the cystoscopic specimens taken from the right ureter fresh smear examinations, pus cells appeared 20 times, white blood cells, 6; epithelium, 22; red blood cells, 18; bacteria, 1. Gram stain,—bacteria, 11; tubercle bacilli were found once. Cultures,—gas tubes sterile, 22; gas positive, 8; cloud and no gas, 8; Endo positive, 5; blood agar plates, sterile, 24, growth, 14. B. coli occurred 8 times, Streptococcus hemolyticus, 1; Staphylococcus albus, 6; hemolytic staphylococcus, 1; Streptococcus pyogenes, 1; B. fecalis alcaligenes, 1.

In the cystoscopic specimens taken from the left ureter fresh smear examination, pus cells appeared 13 times; white blood cells, 7; epithelium, 19; red blood cells, 19; bacteria, 1. Gram stain,—bacteria, 3; tubercle bacilli were found once. Cultures,—gas tubes sterile, 26; gas positive, 3; cloud and no gas, 7; Endo positive, 3; blood agar plates, sterile, 27, growth, 8. B. coli occurred 5 times, streptococcus hemolyticus, 1; hemolytic staphylococcus, 1; Staphylococcus albus, 3; B. fecalis alcaligenes, 1.

The same culture was present in the bladder and both ureters 8 times, in the bladder alone, 13; bladder and right ureter, 9; bladder and left ureter, 1; left ureter alone, 2, and not once in the right ureter. Infection appears most commonly in the bladder alone, next the bladder and both ureters, and is more common on the right side than on the left. The ureters are rarely involved without bladder infection.

The cystoscopic examination was negative in 14 cases, showed evidence of cystitis in 9, involvement of the trigone in 9, obstruction of the ureters in 6, and such other findings as granulation, swelling and thickening of the mucosa, swelling of ureteral orifices, trabeculations, ribbon bands of exudate, mucosal folds, contractions, slough, and the presence of tumor.

The autogenous vaccine was prepared in 16 cases. The organisms used included B. coli, Streptococcus hemolyticus and Staphylococcus albus. They were made from the original cultures as nearly as possible, put up in saline suspension so that 1 c.c. equals approximately five hundred million organisms, killed at 60° C. for one hour and given in ascending doses starting with 0.1 c.c., increasing 0.1 c.c. every other day and continuing for several weeks in 1 c.c. doses. In the stubborn cases where they were of benefit, they produced complete results and, while this paper does not deal with treatment, a vaccine should be tried in every case where other measures cannot be used. One typical case might be cited illustrating this point. A woman, aged twenty-eight, began with frequency, urgency, abdominal pain and pus in the urine of one year's duration. Cystoscope showed distinct evidences of cystitis and the Staphylococcus albus was isolated from the bladder and both ureters. The patient was kept in bed for four weeks with local treatment of boric acid and mercurochrome irrigations. This treatment was supplemented by a vaccine which was faithfully carried out during a further period of five weeks of semirest. Control cultures were made on the bladder which finally became sterile. The patient's symptoms completely subsided; she gained

twenty-five pounds in weight, and has enjoyed perfect health for the past year.

There was a bad dental infection present in 5 cases. This number would undoubtedly have been increased if the teeth of these patients had been examined routinely.

Vaginal smears were positive for gonococcus but once, and a prostatic culture was positive once. The gonococcus plays a small part, if any, in bladder and kidney infections.

Blood serologic tests were made on 45 of the 50 cases; 4 were positive and 41 negative. This percentage is slightly higher than our routine hospital cases, but syphilis seems to have played no direct part as an etiologic factor.

There were 6 operations in the series, 4 nephrectomies, 1 nephrolithotomy, and 1 perineal repair. Except for stone and tuberculosis, the less surgery the better. This series of 50 does not include surgical kidney which is among the larger group.

There are many facts of interest gleaned from these cases which might be profitably included in this paper and set down in a more or less verbatim fashion.

Where B. coli is present in large quantities in the bladder, they may appear in cultures from the ureters although the latter are not involved. This could be determined by the presence or absence of pus cells and bacteria in fresh and Gram smears.

The large number of negative cultures with negative cystoscopic findings and vague symptoms act as a check on the other work reported in this paper.

In a case of pyonephrosis on one side, examination of the corresponding ureteral urine showed marked pus and cultures while the opposite side was perfectly clear. Subsequent nephrectomy proved an infected kidney, and complete recovery proved the other kidney to be normal.

Cases of stone in the ureter may show the presence of much pus from the affected side with sterile cultures.

A case of severe perineal laceration with vague bladder symptoms and B. coli cultures positive cleared up following a repair and no treatment directed at the bladder.

Hematuria is not only a sign of tumor, stone, or tuberculosis, but is present to an alarming degree in hemolytic streptococcus infections. The red blood cells under these conditions are likely to show hemolysis with hemoglobinuria.

Stone in the pelvis of the kidney may be present without any symptoms as in one case where albumin was found at an insurance examination. Subsequent careful study revealed the albumin due to pus, the pus coming from one ureter, and the x-ray revealed the stone.

The gonococcus plays a small part in the series, but it was isolated from the bladder in one case. This was probably more a prostatitis with urine contamination than a real etiologic factor in the bladder.

Where the tubercle bacillus is demonstrated in a twenty-four-hour spec-

imen followed by its presence in one ureter and not in the other, this should be confirmed by repeated examinations before a nephrectomy is advised, because of the frequency in which both kidneys are involved in nephritic tuberculosis.

Several cases with coincident dental infection constitute another link in the study of focal infection and, where cocci are found, teeth and tonsils should always be investigated.

In tuberculosis of one kidney, where pus is pouring out of the infected side with the demonstration of tubercle bacilli without difficulty, and where the mouth of the ureter is granular while the opposite side has clear urine and no ureteral involvement, a diagnosis of unilateral tuberculosis is justified and nephrectomy may be recommended.

Cases of ureteral obstruction following pelvic surgery will show a marked increase in the amount of ureteral epiderm with sterile cultures.

Where urinary system symptoms are present and the case cannot be completely studied for one reason or another, it is impossible to arrive at a diagnosis and unless this case be completed, it had better be dropped.

A case of Streptococcic hemolyticus cystitis failed to clear up under local treatment and vaccine until a pair of bad tonsils were removed and organisms isolated from this source added to the vaccine.

In a case of adenocarcinoma of the kidney, the urine from the ureter on the affected side showed a large number of large unusual cells other than ureteral undergoing necrosis. Such a finding might be considered a suggestive diagnostic point.

A case of ptosis of the kidney, the diagnosis being made by physical signs and the x-ray, gave negative cystoscopic and bacteriologic findings.

In commenting on the high lights of this study, I should say first of all, that complete cooperation between the clinical side and the laboratory must exist with perfect coordination. The material must be received as nearly the same as it is present in the body as possible, and examination must be made immediately.

A simple method of procedure must be worked out in the laboratory. This should include a means of detecting all possible changes which might throw some light on the diagnosis.

The method should differentiate between the bladder and the ureters and between each ureter. It should include a study of the fresh smears, the stained smears (preferably by the Gram method), tubercle bacilli staining, and some forms of routine culture media which will bring out especially B. coli and the cocci groups.

A tentative diagnosis may be made from the bacteriologic studies by the pathologist and, if not a definite diagnosis, at least an interpretation of the findings should be set down. Where this is followed by a conference with the clinician, valuable suggestions come from both sides.

Finally every case is a study in itself and while they fall into general groups and types, many varied pathologic lesions have much in common as to symptomatology and must be completely worked out by the clinician, the

cystoscope, the x-ray and the laboratory. No one group is sufficient unto itself.

DISCUSSION

Dr. A. H. Sanford.—There is one thing I would like to ask Dr. Keilty as to whether he doesn't use the guinea pig inoculation for diagnosis. We find that the inoculation of guinea pigs with urine from each kidney has always been a great aid to the surgeon. We routinely divide the specimen of urine and inject part of it subcutaneously and part of it intraperitoneally. I do not know whether there is any reason for it, but sometimes one is positive before the other one. I would also like to ask what stain he used.

Dr. A. I. Rubenstone—It is a good thing to bear in mind that tuberculosis can be diagnosed in the course of routine urine examinations, especially where many urines are examined. If technicians bear in mind their rule to set aside all pus urines that are acid and of low specific gravity, it is surprising how often one can pick out tubercle bacilli from these specimens when, especially in hospital practice, it might be overlooked. Differential staining by Pappenheim's method has been a great aid in this work. One patient particularly is interesting. A prominent man in the community was told that he had tuberculosis of the urinary tract because acid-fast organisms were found in his purulent urine. When the urine was examined with the ordinary Gabbet's stain, red bacilli were seen in some number but with Pappenheim's rosolic acid stain none could be demonstrated. Further investigation of the patient and guinea pig inoculation proved entirely negative for tuberculosis.

Dr. Frank W. Hartman—We have found that if a method of concentration is used on suspected tuberculous specimens the bacilli are present in sufficient numbers to be demonstrated by almost any of the methods of staining mentioned. The concentration method of Andrus & MacMahan is quite satisfactory.

Dr. H. J. Corper.—I do not wish to talk about staining tubercle bacilli now as this will be dealt with fully in my paper. The subject of examining for tubercle bacilli in the urine is an important one. I personally am not willing to make a definite diagnosis of tuberculosis from the urine unless the microscopic findings are verified by guinea pig inoculation findings, since there are so many possibilities for error in the usual methods. For that reason I prefer to wait in every case before making a definite diagnosis, unless all other evidence points to tuberculosis and the time factor is paramount. It is surprising that many physicians depend implicitly on the finding of a few bacilli in the urine without considering the other pathologic findings in the case or the chances for error in the microscopic method with urine A negative microscopic finding likewise is of very little import because so little material can actually be examined by this method. Our only safeguard to avoid error and make the diagnosis more certain is to inject a guinea pig in which 10 to 100 bacilli can produce tuberculosis, and by means of which all other acid-fast forms, except human and bovine tubercle bacilli, are excluded because they do not produce the characteristic generalized tuberculosis in this animal in from one to three months.

Dr. A. H. Schade.—The guinea pig does not always die; sometimes it is necessary to kill the pig. Many survive and have extensive tuberculosis.

Dr. John A. Kolmer.—I may endorse the statements of the last speaker and believe it always advisable to perform a necropsy on all guinea pigs even when inoculated by subcutaneous injection, since tuberculosis of the internal organs and especially of the spleen, when the external lymphatic glands have apparently escaped infection.

I wish to ask Dr. Keilty his opinion of the possibility of excretion of tubercle bacilli in the urine of individuals without tuberculous infection of the genitourinary organs.

Dr. A. S. Giordano.—About a month ago I had an opportunity to do a postmortem on a patient about sixty-five years old with acute miliary tuberculosis and no pus found in the urine before death. We found tuberculosis of the lungs, spleen, and liver. The kidneys were covered with little tubercles; there was no pus found in the urine at this time nor any tubercle bacilli in the smears, while the pig inoculation was positive.

Dr. Robert A. Keilty (closing).—I did not mention guinea pigs in my paper. I believe that one may tell the tubercle bacilli from experience; I believe that one may find microscopically the tubercle bacilli if one is sure of the stain, etc., and that one may make a diagnosis by the findings under the microscope. I do not see that the guinea pig is important or essential. We all have our own ideas about staining. I believe that rosolic acid is a very good stain. I also use a stain of my own. I do not believe that the tubercle bacilli are in the circulating blood in any great numbers. The possibility of the excretion of tubercle bacilli in the urine is not probably very great although it is to be thought of.

STUDIES IN EMBALMING FLUIDS IN RELATION TO NECROPSIES*

By John A. Kolmer, M.D., and Fred Boerner, V.M.D., Philadelphia, Pa.

WITHOUT entering into a general discussion of the reasons why permission for necropsies is so frequently denied or withdrawn in hospital and private practice, it may be stated that one of them is the objections raised by undertakers. The whole subject has been recently investigated in a most excellent manner by a committee of the Pennsylvania State Medical Society, composed of Dr. Frank C. Hammond (chairman), Dr. V. L. Andrews and Dr. David W. Thomas, who made a survey of the attitude taken by undertakers in opposing the obtaining of consent for postmortem examinations, and in their report[1] it is stated that "the undertaker too frequently urges the family not to give permission, or, if permission has been given, to withdraw it. His reasons for doing so are that: it is impossible properly to embalm a body that has had a postmortem done; it increases the cost of his services; the body will not be turned over to him in 'decent condition'; and, it will not be possible to make the remains presentable. If the proper technic is observed in making the postmortem, no difficulty should be experienced in embalming. The medical profession is to blame in many instances for carelessness in technic and we must correct our indifference if we are to secure the cooperation of the undertaker. If a proper technic is followed, there should be no excuse for the undertaker objecting to an autopsy or making an additional charge for his services. The better class of undertakers will not do so. When a family finds that the undertaker has charged ten to twenty-five dollars extra on account of the postmortem, it will have its baneful influence."

In the hospitals of the Graduate School of Medicine of the University of Pennsylvania we have frequently been denied permission for necropsies on account of this attitude on the part of undertakers, particularly in the case of the burial of individuals of reduced financial status requiring funerals of the minimum expense. Undoubtedly the embalming of a necropsied body requires more time and skill on the part of the undertaker, although as stated by the committee referred to above, the well-trained and experienced morti-

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cian seldom if ever raises any objections and indeed, in our experience, has usually given the most friendly and helpful cooperation. On the other hand it cannot be denied that some morticians will slyly raise objections to the necropsy with the friends or relatives and block permission or even have it withdrawn, despite the assurances of the pathologist to exercise all the usual technical precautions in conducting the necropsy in order to facilitate successful and satisfactory embalming. Among the more important of these technical precautions may be mentioned: (1) To make the routine incision from below the suprasternal notch to the symphysis pubis, except in women, when the incision should not be as high or of the Y-shaped kind, in order to permit the wearing of the customary low neck shroud; (2) to exercise every precaution against undue disfigurement if other incisions are required and to avoid carefully all unnecessary incisions; (3) to keep the circulatory system as intact as possible by ligating all vessels before removing any of the viscera of the chest or abdomen; (4) to examine the aorta *in situ*, but if removed, to ligate the left carotid and innominate arteries, leaving eight inches of ligature attached; in other words to tie off all large arteries and veins severed in doing the work after the blood has drained out. (The vessels tied are the following: The right innominate, the left carotid, and the left subclavian, and the two innominate veins in the neck; the renal arteries, the celiac axis and the superior and inferior mesenterics. In case the aorta is removed to tie both femoral arteries and veins.) (5) To ligate the rectum below the line of incision if the intestines are removed; (6) to remove the brain by a scalp incision from ear to ear over the vertex and to prevent leakage from the carotid arteries by plugging, or tying off at the base of the skull, with plaster of Paris or other means; (7) to keep the head and shoulders raised during the examination in order to allow the blood to drain away; (8) if the body is turned face downwards for removing the spinal cord, to protect the face and especially the nose, against bruising; (9) to sponge out all blood from the cavities and pack adequately, and (10) to sew up tightly and neatly and remove all blood stains. In addition to these precautions of considerable importance to the mortician, all of them reasonable and readily complied with, the pathologist should show him the courtesy of delivering a clean body as nearly as possible at a specified time. Sometimes it is considered advisable to permit the undertaker to embalm the body before the incision is closed and especially in cases where extensive removal of viscera has been done.

In small communities where the pathologist and morticians may be personally acquainted and an understanding on these points reached by working together and in harmony, difficulties are not likely to arise, but in a large city it may be otherwise, and the fact remains that not a few of the so-called little undertakers lose no time in obtaining immediate possession of the body in order "not to lose the job," and in Philadelphia at least some will not hesitate to advise the friends and relatives against permission for a necropsy or to have it withdrawn in order to get the "job" away as quickly as possible and to minimize the amount of work and skill required for preserv-

ing the body for the funeral. Furthermore, all undertakers are anxious to embalm as soon after death as possible in the interests of satisfactory preservation of the body.

Such being the circumstances, it has occurred to us and doubtless to many other pathologists who have lost necropsies on account of this attitude on the part of some undertakers (but probably sometimes justified by carelessness and lack of courtesy on the part of the pathologist) to give more attention to the possibility of inviting the undertaker to embalm first and to provide him with an embalming fluid if necessary along with adequate working facilities. By reversing the usual order in this manner we may at least remove his objections even though it is to be realized that they have but little real justification at best, providing the pathologist is willing and capable of observing the above-mentioned precautions.

One insurmountable objection to this plan, however, is that bacteriologic cultures cannot be made after embalming; chemical analyses of organs or the gastrointestinal contents may likewise be disturbed or rendered entirely useless, but the latter are required in but a very small percentage of cases and in reference to the former, means may be made when bacteriologic examinations are required. In so far as cultures are concerned, embalming fluids invariably possess a high degree of bactericidal activity to fulfill their functions, rapidly sterilizing the blood and fixed tissues with which they come in contact.

As all pathologists of experience are aware, a fairly satisfactory necropsy can usually be made on an embalmed body in so far as the detection of the gross pathologic lesions are concerned. It is true that the organs and tissues may be rendered tough, leathery and blanched, by the formalin incorporated in almost all embalming fluids, or discolored by red dyes, which may render difficult or impossible the macroscopic detection of the finer tissue changes, but the detection of the gross changes is usually quite possible, most difficulty being experienced in the detection of embolism and thrombosis, since the usual embalming fluids quickly convert residual blood into tough gummy masses.

As we have observed in numerous experiments with various embalming fluids to be found in the market, the histologic examination of embalmed tissues is oftentimes difficult, especially from the standpoint of vascular changes, since the undertaker does not remove all blood from the internal organs by his usual technic and the sections almost invariably show extensive congestion and some interstitial hemorrhage.

From the standpoint of the mortician an embalming fluid must serve the following fundamental purposes: (1) Preserve the body against putrefactive changes for at least the usual period; (2) fix and harden the tissues and at least the muscles of the face to prevent dropping of the jaws when the rigor has passed away, and (3) as far as possible, replace the pallor of death with some more lifelike color, especially the skin of the exposed parts. To secure these ends he must inject sufficient fluid for the preservation of the skin, and it is imperative that the fluid injected shall be sufficient for preserving the

body after the necropsy has been done, although it is readily possible for the mortician to reenforce his efforts by injecting additional amounts of embalming fluid into the thoracic and abdominal cavities, subcutaneously or even into the great vessels of the neck or any particular arm or leg, after a complete necropsy has been conducted.

From the standpoint of the pathologist the ideal embalming fluid for injection before necropsy should possess the following fundamental properties: (1) It should not discolor, harden or otherwise alter the physical state of the organs and tissues and (2) it should not alter the residual blood. To these may be added a third property, it should not contain the usual poisons like arsenic, in case chemical analyses are required in medicolegal cases. One is tempted to add that it should not be bactericidal in order that bacteriologic examinations of the blood and internal organs may be made during the necropsy, but this is superfluous when it is remembered that preservation demands that the embalming fluid shall be parasiticidal or at least parasitostatic in action.

During the past two years we have experimented with a number of embalming fluids in common use among undertakers and found all of them to contain so much formalin that when used in the dilutions and amounts recommended, the internal organs were changed in color and consistency with marked changes in the microscopic pictures involving mainly the residual blood. Indeed, some of these fluids contain so much formalin that it is impossible to distribute it evenly through the capillary tree, by reason of producing vascular occlusions, and one cannot escape the conclusion that some morticians depend largely upon the principle of getting sufficient into the great vessels and abdominal cavity to take care of the balance of the body for a few days at least by gaseous distribution of formaldehyde. In discussing this subject Robertson[2] of the Mayo Clinic, possessing a very extensive necropsy experience, states that if the undertaker will use his solution in one-half the usual strength, he will wash out blood more easily, obtain a more intensive penetration of the capillary tree and that he will be more pleased with the results than he would be with his stronger solution, although if the cheeks are not fixed tightly enough he may find it necessary to inject the head with the usual strength solution. Our experiments have amply confirmed Robertson's statements and it is advised that when embalming is done before the necropsy is conducted, but the undertaker be requested to dilute his usual fluid one-half more for injection into the body below the head but that for the head he use the usual strength solution.

But from the standpoint of the pathologist the conditions are not yet just right and the mortician is sometimes required subsequently to take care of patches of skin showing discoloration before burial. From the standpoint of preservation we have had much better success with an embalming fluid prepared by Dr. Addinel Hewson,[3] Professor of Anatomy in the Graduate School of Medicine in the University of Pennsylvania. This fluid contains only one and one-half per cent formalin and therefore permeates the capillary tree very readily and easily, does not cause marked changes in the

physical state of the organs and alters but slightly or not at all the usual histologic appearances. Furthermore, it has been a very satisfactory preservative since we have kept necropsied dogs at a temperature of about 24 degrees C. for weeks and indeed, for months, without putrefaction. Unfortunately, however, it does not harden the muscles of the jaws sufficiently and the low formalin content would make it illegal for embalming purposes in some states.

At this stage of our investigations we were invited by Dr. Hammond, chairman of the aforementioned committee of the Pennsylvania State Medical Society, to confer with Mr. Frank K. Fairchild of the National Selected Morticians Research Corporation, and it is a great pleasure to record here the instant response and hearty and most helpful cooperation of Mr. Fairchild and their consulting chemist, Mr. Jerome Alexander of New York. Several delightful and helpful conferences with these gentlemen have aided us greatly in understanding and appreciating the difficulties of the situation and to make clear to us the very earnest efforts being made by the National Selected Morticians to improve the professional education of morticians and to seek and merit the closest cooperation of the medical profession. We are indebted to Mr. Fairchild and Mr. Alexander for a number of embalming fluids for our studies and especially for the privilege of experimenting with one of their own manufacture to be rendered available for use by undertakers; the proceeds from the sale of which are to be devoted to the promotion of research work on embalming fluids and methods. This fluid contains formaldehyde, glycerin, borax, citrate, sodium chloride, potassium nitrate, benzaldehyde, and alcohol. By a slight modification in their original fluid, consisting of a reduction in the glycerin content and an increase of the alcohol, we have embalmed numerous dogs followed by necropsies at varying intervals and found the fluid, injected in proper amounts according to body weight, capable of preserving the bodies for weeks and even months at room temperature with remarkably slight alterations in the gross and histologic appearances of the internal organs and muscles.

Without detailing the results of our investigations at this time we believe that it is readily and easily possible for the physician to meet all the legitimate objections to the necropsy raised by the mortician either by conducting the necropsy with the technic and courtesies summarized above or by asking the mortician to embalm before the necropsy. If the latter procedure is adopted we are in position to state that *if the undertaker will do his work thoroughly in order to obtain a wide distribution of the embalming fluid throughout the capillary tree, he need not worry at all about preservation* and that for this purpose he may use either his usual embalming fluid diluted one-half the usual strength (with full strength for the head) or one of the newer fluids, like those prepared by Professor Hewson and Mr. Alexander. It is true that the pathologist may encounter the fluid in some of the great vessels and serous cavities but this need not be confusing; in so far as the purposes of the necropsy are concerned its ends may be readily attained and the mortician and friends of the deceased may be assured that the preservation

of the body will not be interfered with, providing the undertaker injects the embalming fluid with a reasonable degree of skill and thoroughness. If the mortician will do his work well and the pathologist conduct the necropsy with reasonable skill and care and be not lacking in sympathy and courtesy toward the mortician, there need not be any excuse for a lack of heartiest cooperation between the medical and undertaking professions, with an increased percentage of necropsies so vitally necessary for the advance of medical science and education.

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DISCUSSION

Dr. Alexander.—There is a movement on foot to make a real profession out of undertaking. I do not need to tell you that the great profession of medicine was once not all that it is today. In the last few decades it has developed most remarkably. Compared with early times, there has been a very remarkable change in funeral customs. In ancient Egypt the embalmers were required by law to report to physicians anything they found out in the course of the embalming process, in order to advance the science of medicine. It is highly important to remember that the care given to the dead has a beneficial effect on the living.

Dr. Kolmer has been put in possession of the facts concerning this movement to make all undertakers cooperate more readily with physicians. I hope that as a newer class of undertakers develops, professional ideals will be more widespread among them, and physicians will find that they will get more useful cooperation from embalmers. I wish to thank Dr. Kolmer for the work he has done in this matter.

Mr. Papello.—The subject has been very well covered by Dr. Kolmer and Mr. Alexander. There is no doubt at all but that the real progressive mortician wishes to cooperate with the physician. There is no reason why any real undertaker should not be glad to cooperate.

Dr. A. S. Giordano.—I wish to congratulate Dr. Kolmer on his paper. When I first came to South Bend I was very much surprised to find the autopsy percentage in hospitals almost zero. Necropsies were opposed by morticians. We got together with the undertakers and the autopsies have increased. I am very much in favor of not having any embalming done before autopsy for very obvious reasons, and, as a matter of fact, embalming can be done more easily and more completely after the autopsy than before. Therefore, to obtain complete cooperation from the morticians, the pathologists must take the trouble to demonstrate this point to his morticians, and then all opposition will cease.

Dr. Margaret A. Miller.—As far as the undertaker is concerned, I should think it would be easier if all of the organs were removed. On the other hand, many times relatives object to having any of them removed. Frequently something is found in sections which might give a solution of the problem if it were possible to reexamine the entire organ, and take sections along the course of the ducts or vessels.

Dr. Eugene A. Case.—If we remove organs when we have been asked not to, we lay ourselves open to the charge of dishonesty. The end sought does not justify the means. The question of the use of embalming fluid is an important one. In an autopsy your findings are based upon the consistency of the organ, its shape, its size, its color, the amount of blood present or the absence of blood, etc., and embalming may so change the appearance of the organs that it will be difficult or almost impossible to form a correct opinion of the pathologic alterations present, this process of embalming destroying much valuable evidence. In certain diseases we may wish to apply special stains to the cells, and as most embalming fluids contain formaldehyde this becomes exceedingly difficult. We are confronted, however, with the condition that whether or not we so desire we must cooperate

with the undertaker; therefore where we find that an autopsy can be secured only by permitting the embalming to be done first, we should permit the embalming.

Dr. T. H. Boughton.—The necessity of not removing organs, together with the obligation of cooperating with the embalmers, led me to develop a very simple method that is applicable in ninety per cent of the cases. With the exception of the brain and the heart, practically any organ can be examined without removing it from the body, simply by lifting it up. It is not necessary to remove any organ or to sever relations with the organ.

Dr. Ralph G. Stillman.—I think it is no more than right that we should pay our respects to the committee of the Pennsylvania State Medical Society for its work with the morticians. When we showed the recommendations made in this report to some of the undertakers and talked it over with them, we found that relations were much improved. So far as the question of autopsy as a whole is concerned it seems to me that it is necessary to work not on the morticians but on the State Legislature. In New York the law has provided that any autopsy shall cease immediately when cause of death is discovered, which taken literally, would terminate many autopsies before they are really completed. It is necessary to remove organs for microscopic examination. It has been impossible to get autopsies where the nearest of kin was at a great distance; the law now provides that the Superintendent of the Hospital may order an autopsy to be performed in the absence of objection on the part of the nearest relative.

Dr. John A. Kolmer (closing).—If the organs are removed the question of their preservation depends a good deal on the period of time intervening between the time of embalming and the time of autopsy. If the autopsy is done immediately it is entirely probable that some of the embalming fluid will escape. The organs are ordinarily returned to the abdominal and thoracic cavities. In regard to the second question, I am sorry that I have no accurate data to draw upon. I am of the opinion that when the necropsy is to be done, it should be done as soon after death as possible, the body in the meantime being kept at a low temperature.

SEDIMENTATION RATE OF RED BLOOD CELLS*

By H. N. Cooper, M.D., Watertown, New York

THE sedimentation rate of the red blood cells is a phenomenon which has attracted but little attention in this country. On the other hand, in reviewing abstracts of foreign journals, especially the German ones, one is impressed with almost weekly references to this test. The startling variations in the rate at which the red blood cells settle down in oxalated blood, when left standing, seems to indicate that definite conclusions could be drawn from this observation, and sufficient work has been done on the test, corroborated by many workers, to place this test in more common usage in all laboratories. Valuable information can be placed at our disposal in many instances.

METHODS

Westergren[1] used a method which has been copied somewhat but which required special syringes and tubes and seemed unnecessarily complicated. Linzenmeier[2] noted the time required for the red cells to settle to definite points. His technic is used in many instances and a recent paper by Baer and Reis[16] of Chicago commends this method. Fahraeus[3] used multiple readings at definite time intervals, which feature I have copied. Zeckwer and Goodell[4] in this country used 8 c.c. blood and 2 c.c. oxalate solution and read at one hour and twenty-four hours. I have modified their technic in two ways: (1) using powdered oxalate, thus avoiding dilution, and (2) multiple readings within the first hour, which seem to have an advantage. Blood is drawn into a bottle into which three drops of 20 per cent potassium oxalate has been allowed to dry. This anticoagulant is sufficient for 15 to 20 c.c. of blood and is used in conjunction, usually, with the taking of a specimen for blood chemistry. The test should be performed as soon as possible, although a delay of a few hours does not materially change the readings. Into a dry 15 c.c. graduated test tube is put 5 c.c. of blood. This is allowed to stand in a rack and readings are taken of the level to which the cells have settled at the following periods: 5, 10, 15, 30, 45, and 60 minutes. The tube is centrifuged at high speed for ten minutes and a reading is taken, which corresponds to the cell volume. This figure is practically identical with that obtained by twenty-four hours' sedimentation. During the latter part of my work, the readings were figured in per cent; thus a cell volume of 2.5 c.c. (of 5 c.c. blood) is 50 per cent. While a confusion of tests is undesirable, I feel that this method is as simple, as free from extraneous influences, and gives as complete a curve as any of the above methods.

*From the Department of Laboratories, House of the Good Samaritan, Watertown, New York.

Read before the American Society of Clinical Pathologists, Philadelphia, Pa., May 22, 1925.

NORMAL STANDARDS

All cases, excepting the four groups below mentioned and those with a red blood cell count below 3,500,000, were used to determine the normal levels. Fifty-eight cases are used in Chart I, showing the range within which these fell. The fastest rate or the lowest line in this group determines the division between the normal and pathologic. The importance of a conservative idea of the normal is obvious. In over one hundred and twenty-five tests every reading below this line was definitely pathologic and could be classed in one of the four groups.

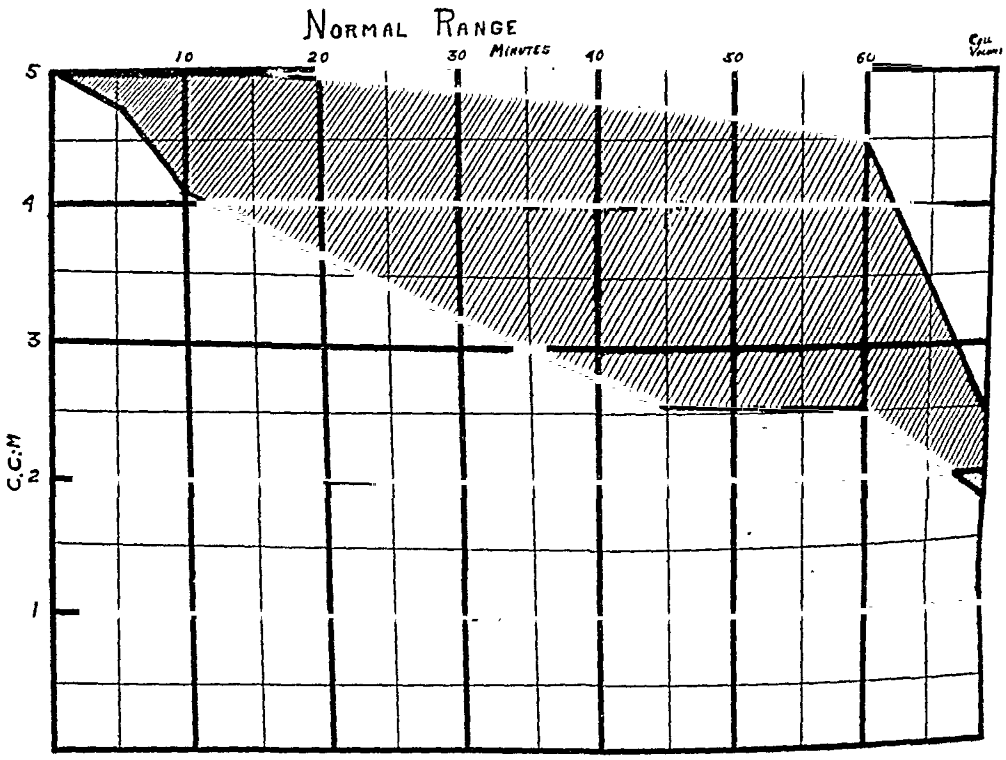

Chart 1

ACUTE INFLAMMATIONS

Chart 2 lists six cases. A definite acceleration is noted in all except line 1, in which case the acute inflammation had subsided, the cause being removed at operation and presumably the rate having returned to normal even before the white blood cell count had subsided. Definite conclusions cannot be drawn from the few cases recorded here, but, in conjunction with other reports, the conclusions are drawn that the line lowers as the leucocytes increase, and a lowering in the early periods corresponds to a high polymorphonuclear count and gives a good prognosis. This rapid fall in the cells in the five and ten minute periods is very striking and, so far in our cases, indicates a favorable reaction. The cell volumes in these cases were nearly normal. Linzenmeier,[2] in reviewing many tests done on gynecologic cases, states that as long as the rate is increased virulent bacteria may be present

in the body, and, hence, he advises delay in operation. He also uses this test to differentiate between acute inflammation of the adnexa and tubal pregnancy, the latter showing no increase. Baer and Reis[16] in a recent and very creditable work, show the value in pelvic pathology of this test, both as a means of differential diagnosis and as a prognostic index.

TUBERCULOSIS

A great amount of work has been done with this test in connection with tuberculosis. Morriss[5] says this test offers a valuable estimation of the degree of activity of this disease. Patients with definite symptoms of activity, fever,

ACUTE INFLAMMATION

Chart 2

Acute Exudative Inflammations

No. 1.—Case 61. Acute cholecystitis four days postoperative. W.B C 16,000
No. 2.—Case 36. Erysipelas, first day, blood taken before eruption appeared. W.B C. 15,000.
No. 3.—Case 43. Abscessed appendix. W.B C. 10,400.
No. 4.—Case 46. Pelvic abscess.
No 5—Case 51. Bilateral pneumonia, empyema, died.
No. 6.—Case 67. Lobar pneumonia, recovered.

and rapid pulse, always give an increased rate. Popper and Kreindler[6] examined two hundred and fifty patients and found that all progressing pulmonary affections show accelerated sedimentation, even with minimal lesions not yet discoverable on clinical and radiographic examinations. Gardère and Lainé[7] made a study which showed a parallelism between a rapid rate and the gravity of the disease. Dreyfus[8] agrees with this and thinks this test is a

better indication of the activity of the process than the temperature curc. Weicksel,[9] however, is cautious in his prognosis except in advanced cases. As shown on Chart 3, minimal cases or those improving show a normal rate, and the worse the involvement the faster the fall in the level of cells. The cell volume bears a constant relation to the patient's general condition. A high value is found in cases progressing favorably. In this our records agree with those of Schlomovitz.[10]

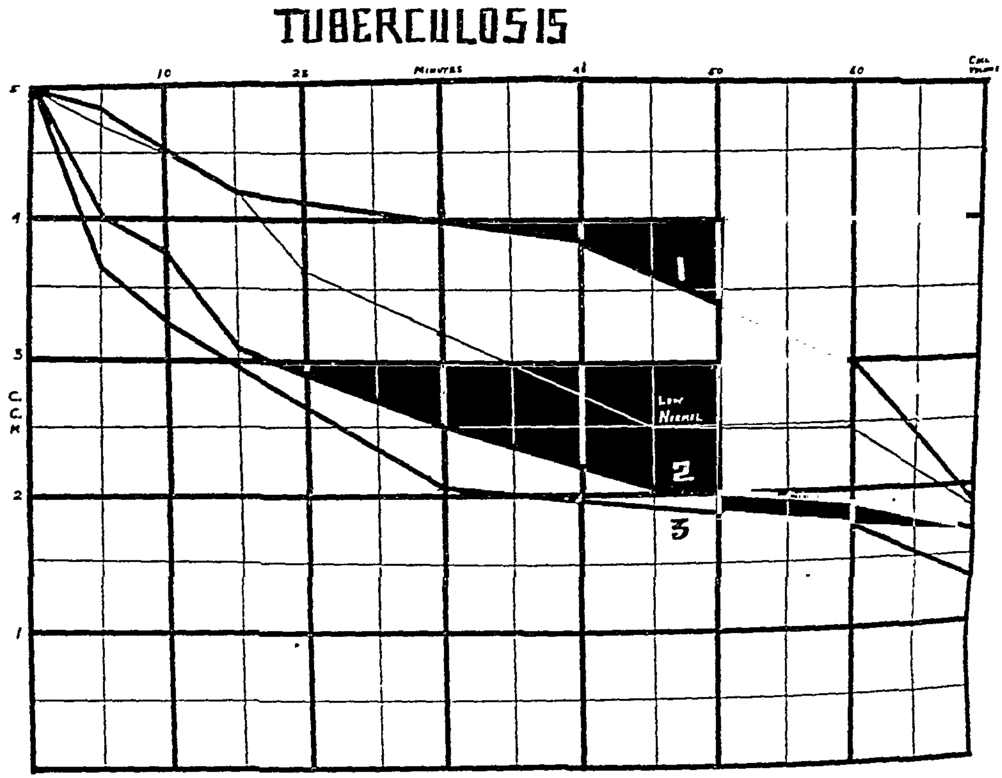

Chart 3

No. 1.—Case 76. Bilateral tuberculous epididymitis two years ago. Osteomyelitis of ribs. Age 83. Condition good.
Case 100. Incipient tuberculosis. Temp. 99.
Case 101. Minimal tuberculosis. County sanitarium.
Case 104. Positive sputum. Gained weight. Improvement.
No. 2.—Case 103. Moderate pulmonary and laryngeal tuberculosis.
Case 102. Moderate pulmonary. Temp. 102.
Case 66. Sputum positive. Temp. 103.
No. 3.—Case 12. Pulmonary tuberculosis, diabetes, died.
Case 98. Advanced sanitarium case.
Case 99. Terminal sanitarium case.

PREGNANCY

Pregnancy shows an increased rate. Pewny[11] shows that during later pregnancy his test will differentiate between that condition and myoma. Zeckwer and Goodell[4] noted that some cases of pregnancy did not have an increased rate. In Chart 4 I have grouped the cases according to the period of gestation and the presence of toxic symptoms. There is a progressive acceleration during pregnancy, and the presence of even slight toxic symptoms, such as morning sickness, increases the rate in that period. One of

the fastest rates in this series of cases is shown in line five; it is a combination of pregnancy, toxemia, and acute inflammation. Briefly, the patient presented on admission the picture of eclampsia, acute nephritis with complete suppression of urine, delivered of a dead fetus, high blood pressure and a rapidly increasing nitrogen retention of the blood. A decapsulation of one kidney was done and following this, urine began to be excreted in increasing amounts. The sedimentation test showed a marked acceleration on the day of her operation. The fastest rate was on the day she started excreting urine.

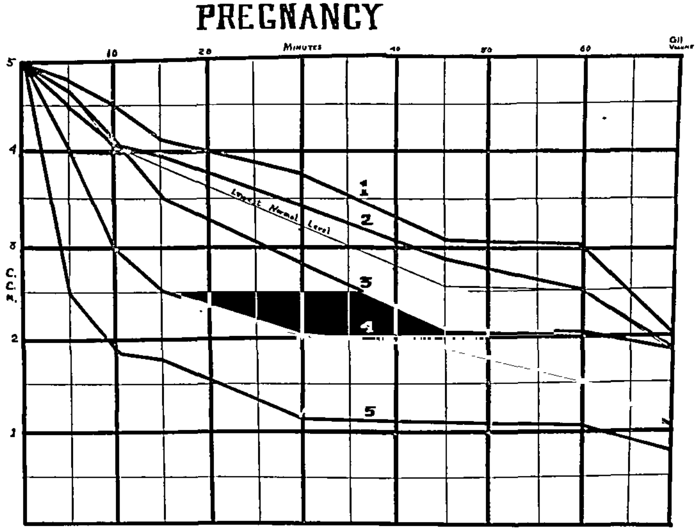

Chart 4

No. 1.—Average of seven cases. Normal pregnancy, three to seven months.
Case 29 3 months pregnant, normal.
Case 41. 6 " " "
Case 84 4 " " "
Case 87. 4 " " "
Case 78. 7 " " "
Case 91. 6 " " "
Case 92. 3 " " "
No. 2.—Average of three cases, showing early toxemia.
Case 79. Nausea persisting for six months.
Case 85. Nausea, four months pregnant.
Case 86. Five months, nausea and slight edema.
No. 3.—Normal pregnancy over seven months.
Case 33. Normal pregnancy at term.
Case 49. Normal at eight months.
Case 60. Nine months, normal except for four-plus Wassermann.
Case 89. Normal eight months pregnancy.
Case 90. Normal seven months pregnancy.
No. 4.—Late pregnancy, moderate toxemia.
Case 42. Nausea persisting, edema, urine normal.
Case 93. Urine shows trace of albumin, blood pressure 160/85.
Case 97. Albumin in urine, blood pressure 145/90, labor induced.
No. 5.—Eclampsia, acute suppression of urine, N.P.N. 170 mg., creatinine 6.6, cholesterol 532. This line is the lowest line (fastest rate) in the series and was taken at the highest point of the disease. Three other readings showed a slower rate when the disease was less severe. Patient recovered.

3. The cause of the increased rate is associated with a hypercholesterolemia as well as an increase in globulin and fibrinogen in the blood.

I wish to acknowledge my thanks to Dr. Page Thornhill, Chief of the Pre-Natal Clinic and to Dr. H. L. Smith, Director of the County Tuberculous Sanitarium, for their aid in securing material for this work.

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DISCUSSION

Dr. Ward Burdick.—I know nothing about it, but I have noticed this phenomenon several times while doing fragility tests, not knowing how to interpret it. I am very glad to have heard this paper. It apparently opens up a field full of promise.

Dr. S. L. Leiboff.—We have obtained similar results to those of Dr. Cooper and in our technic we simply use one c.c. of blood instead of ten. We found that by using 1 c.c. of blood we get just as good results. We read the tubes at five minutes, ten minutes, fifteen minutes, half an hour, and two hours.

Dr. Robert A. Kilduffe.—I think this is an extremely interesting paper and one which I had hoped to see more freely discussed.

This method of investigation is applicable to and of interest in a great variety of conditions, but, in order to have conclusions of value drawn, some idea of the normal variations must be had.

How is this to be obtained, however, if everyone using the test employs a different method? The method described has the advantage that the specimen may also be used for blood chemistry.

I think it would be very important and of value if the members of this Society would investigate different methods and the use of different amounts of blood so that we could determine, first of all what was a satisfactory amount of blood, and then, perhaps, using one method, we could get figures which would be comparable and of definite value.

Dr. George D. Fussell.—I would like to ask if an anemia does not alter the reading a great deal, regardless of the cause of the anemia.

Dr. H. N. Cooper (closing).—As I mentioned, everyone is using a different method, each seeming to favor his own modification. In regard to the reasons why I did not use Westergren technic: first, the syringes, etc. are hard to obtain and, second, I feel inclined

not to favor small tubes, anything less than 5 mm. in diameter, as capillary attraction and other elements enter into the reaction in such cases. A large tube, it seems to me, is more desirable. The reading, as indicated in the early part of the first hour, has a great deal of significance, and, while other observers have used frequent readings, the majority have read at hourly periods. Diluting the blood with oxalate solution may have something to do with the sedimentation rate. This test obviates that factor, and, further, the blood can be used later for blood chemistry determinations. Anemia does not increase the rate. There is no doubt that a standard test would be desirable, and I think it would be a splendid thing for this Society to establish a standard for this test.

SUGGESTED METHOD TO BE FOLLOWED IN DEVELOPING A STANDARD COURSE FOR MEDICAL TECHNICIANS*

By Walter E. King, M.A., M.D., Detroit, Michigan

THE clinical laboratory is more than a workshop involving mere matters of routine. It represents a clearing house, to which is referred clinical materials of widely diversified nature. From the studies of the materials subjected to examination, data are obtained which are utilized by the physician. As Woolley has stated, "By means of such data the physician is more easily able (or more quickly able) to arrive at a diagnosis, to estimate accurately a prognosis, and to institute confidently logical therapeusis."

In efficiently conducting the various analyses literature must be consulted, judgment must be exercised, experience and training must be called into action. In the small clinical laboratory, under the supervision of a visiting pathologist, as well as in the larger, well-organized laboratory directed by a resident clinical pathologist, one or more individuals are called upon to do this work. These individuals are regarded as medical technicians. A medical technician is one who is able to conduct clinical laboratory tests, examine clinical materials, and assist the pathologist and the physician in carrying out various diagnostic procedures.

Heretofore, relatively scant attention has been given to the part which the medical technician takes in the diagnosis of diseases. The technician is, of course, required only to report various findings to the pathologist; such work, however, carries with it a great responsibility. Unless the technician's work is carried out intelligently and carefully the clinician's diagnosis is in danger of being inaccurate. Just as the physician's clinical diagnosis should be based upon thorough scientific observation, resting upon a background of honesty of character and knowledge of medicine, so must the laboratory reports be based on careful and intelligent work where reliability is vouched for by a trained technician.

The purpose of the laboratory is to discover facts which will help the physician and benefit the patient. No degree of skill, knowledge, experience, or brilliancy of any laboratory director, or clinical pathologist can entirely

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From the Medical Research and Biological Laboratories, Parke, Davis and Company, Detroit, Michigan.

compensate for lack of skill, scanty knowledge, inexperience, or stupidity on the part of the technician.

At the present time, the maintenance of laboratory facilities or access to such has become a necessity to every community. Hospitals, clinics, physicians, individually and in groups, sanatoriums, asylums, private and industrial laboratories, and state and municipal boards of health laboratories must be supplied with more and better trained technicians. The work which these individuals perform is as exacting and as highly professional in nature as that of the registered pharmacist or the registered nurse.

Fifty years ago the clinical laboratory, as an important diagnostic aid, did not exist. Today we are confronted with the problem of the training of individuals for this comparatively new profession. We must, therefore, keep in mind, first, the rapid development of medical technology and, second, the rapidly growing demand for more and better trained men and women in this vocation.

A variety of methods has been followed in attempting to train laboratory technicians. The most common procedure has been to train the worker individually. One frequently hears a laboratory director state that he prefers to train his own technicians. Such training must necessarily be relatively narrow and result in too great a degree of imitation. Unfortunately some technicians who might otherwise have been good students are spoiled by this method and become inefficient, automatons rather than broadly trained, helpful assistants.

Too many of our prominent clinical pathologists and physicians have voiced the statement that any experienced laboratory man can train technicians to do the routine work. The director of a busy laboratory has little available time to devote to the development of his assistants. Even in the presence of the leaders among the American clinical pathologists, one may boldly make the statement that not all clinical pathologists are teachers. Perhaps at a somewhat safer distance one might venture the remark that few clinical pathologists possess the patience, the love of imparting knowledge, and the passion for stimulating development that are required of the successful teacher.

There are many who believe that the college or university course offers all that is necessary for the student who desires medical technology as a vocation. This method of training has been tried for many years. Regardless of the courses which are elected, the student finds himself at the end of the college or university career almost totally lacking in practical knowledge and laboratory experience in the most commonly required diagnostic procedures. This criticism applies, to a very considerable extent, even to those universities which offer certain courses recommended as leading to service as clinical laboratory technicians. Making proper allowance for the broadening influence and the added advantage of college education for any line of work, however, one may observe almost as many failures among technicians who are college graduates as among those who have attempted to pick up training. The reason for this lies in the fact that the successful medical technician needs not only an intimate working knowledge of the various subjects involved, but also requires *a correct viewpoint of medical technology as a pro-*

fession. The student should secure a broad foundation, learn well the many different methods of technic, become proficient in scrutinizing newer developments, understand the interpretations of laboratory findings, and learn to appreciate the doctor's problems. In other words, *adequate preparation for this work involves professional training.*

There has rapidly developed a necessity for a prescribed course of study consisting of didactic teaching, laboratory exercises, and practical training, all carried out according to strict pedagogical methods. The ideal training for medical technicians should consist of a four year course of study leading to the degree of B.S. in medical technology. As has been stated, such a course cannot be secured advantageously by selecting subjects here and there from different departments of the university or medical school. It is essential that such a course should be strictly technical or professional in which a large proportion of the work is that specifically involved in medical technology.

To develop the proper course of training for medical technicians the work must necessarily pass through transitional stages. The extent of demand for technicians, the perfection of new methods of laboratory diagnosis, as well as other factors, would materially influence the trend of such development and the period of time necessary to reach the point when a standardized course of training might be realized.

In the plan which is outlined, it is assumed that this project would be undertaken under the direction of a medical school, preferably one which is a part of a large university. The institution should be closely affiliated with hospital facilities and, if possible, with the local state Board of Health laboratory.

Such would be the ideal arrangement. Any educational institution of good standing, however, could put into practice the plan which is submitted and thereby fulfill a distinct service to the medical profession and to humanity.

The courses which are outlined would be subject to considerable change, as the aim should be to give proper balance, emphasizing those subjects most demanded of the laboratory technician. No diagnostic procedures should be omitted. Those who are interested in teaching these students find it necessary to make frequent and sometimes radical changes in the texts of laboratory directions in order that proper instruction may be maintained. The student should be impressed with the rapidly changing methods of the laboratory field and should possess a training broad enough to recognize and adopt newer methods of procedure which are of value. This constitutes one of the most important factors in the successful training of the student technician and establishes in a definite manner the need for a well balanced, pedagogically conducted, technical course of study for clinical laboratory workers.

Conservative judgment would indicate that a school for student medical technicians, which would fit those capable of doing accurate work and of growing in the field of endeavor, could be well developed within a period of from three to five years.

It would seem best in the beginning to offer only a vocational course. This could be extended as rapidly as conditions would justify, and within a few years the institution, which might undertake this project, should offer a four year course carrying with it a Bachelor's degree for those who might

desire the full college course, and a two year course, vocational and technical in character, not leading to a degree, for those wishing to qualify as certified routine workers only.

Courses of study should be arranged under three divisions, the three divisions representing stages in the development of a standardized course of study for medical technicians. Stage one, which might be undertaken in the beginning and which would consist of a full one year course, should occupy for its development a period of only one year, certainly not more than two years. Stage two, consisting of a full two year course, should be well begun during the third year after the beginning of the project. Stage three, representing a four year course, leading to the Bachelor's degree, could be instituted at any time that conditions might justify.

In the following suggested plan, it is assumed that all routine laboratory diagnostic procedures are included under the various courses listed. For instance, the routine examination of spinal fluid is included under "clinical analytical methods," "serology," and "hematology."

The various courses submitted are suggested as a basis upon which to build. As the work progresses, some additions and considerable rearrangement probably would be found necessary or desirable.

The outline here submitted is the result of three years of intensive study and practical application in the training of approximately one hundred and fifty student technicians.

STAGE I

Prerequisite: High school course, from schools accredited by the Association of American Colleges.

One Year Course, covering a period of nine months (36 weeks or 1080 college hours) of didactic training, followed by four months (16 weeks, 8 hours per day, approximately 700 working hours) of apprenticeship or practical training in an accredited hospital or public health laboratory.

The one year course may be arranged as follows, and cannot include instruction in roentgen ray or basal metabolism except through a few "special lectures."

Arrangement of Courses:

SUBJECT	LECTURE HOURS	LAB. HOURS	LAB. PERIODS (4 HR.)
Clinical Bacteriology	48	136	34
Clinical Analytical Methods	48	120	30
Hematology	30	120	30
Serology and Immunology	34	148	37
Blood Chemistry	20	40	10
Urinalysis	30	80	20
Parasitology	15	8	2
Tissue Technic	5	40	10
Functional and Sensitization tests	3	28	7
Anatomy and Physiology	40		
Medical Terminology	40		
Interpretation of Findings	10		
Autopsy Demonstrations	10		
Special Lectures	27		
Total	360	720	180
Apprenticeship (16 weeks, 8 hours per day)		704	
Total laboratory		1424	

STAGE II

Prerequisite: High school course, from schools accredited by the Association of American Colleges.

Two year course, covering two nine month (36 weeks or 1080 college hours) periods of didactic training, with two four month (16 weeks, or approximately 700 working hours) periods of apprenticeship or practical training in an accredited hospital or public health laboratory.

SUBJECT	LECTURE HOURS	LAB. HOURS	LAB. PERIODS
Clinical Bacteriology	72	200	50
Clinical Analytical Methods	72	200	50
Hematology	48	180	45
Serology and Immunology	60	220	55
Blood Chemistry	32	80	20
Urinalysis	32	88	22
Parasitology	20	20	5
Tissue Technic	18	80	20
Functional Tests	10	20	5
Anatomy and Physiology	72	8	2
Sensitization Tests	6	4	1
Roentgen Ray	120	240	60
Basal Metabolism	26	100	25
Medical Terminology	72		
Interpretation of Findings	10		
Autopsy Demonstrations	10		
Special Lectures	40		
Total	720	1440	360
Apprenticeship (32 weeks, 8 hours per day)		1408	
Total laboratory		2848	

It is suggested that this course be divided as follows:

First Year:

SUBJECT	LECTURE HOURS	LAB. HOURS	LAB. PERIODS
Clinical Bacteriology	72	220	55
Clinical Analytical Methods	72	220	55
Hematology	48	180	45
Urinalysis	32	88	22
Anatomy and Physiology	72	8	2
Sensitization Tests	6	4	1
Medical Terminology	50		
Special Lectures	8		
Total	360	720	180

Apprenticeship (16 weeks in urinalysis, blood, and general routine work including clinical bacteriology).

Second Year:

Remainder of subjects and completion of apprenticeship (16 weeks) emphasizing Roentgen ray, blood chemistry, serology, and basal metabolism.

STAGE III

Prerequisite: Same as above.

Four year course, leading to Bachelor of Science degree in Medical Technology. This course should consist of the two year technical course presented above, preceded by two years of college work. The college work should consist of the accepted premedical course of sixty semester credits of college work, including English, six credits; Chemistry, thirteen credits; Physics, eight credits; Biology, six credits; and French or German, six credits. Subjects suggested or advised are Latin, English, Mathematics, Psychology, Sociology, Physiology, Comparative Anatomy and Drawing.

It can be seen that such a scheme, with such modifications as conditions required, could be undertaken without difficulty at any well organized educational institution. Insurance against failure would be afforded by a natural evolution of the training from a one year vocational course to a four year college course. Students successfully completing such courses, trained in an academic atmosphere, inspired with true scientific spirit should promptly find a large measure of usefulness.

Such a project, successfully carried out, would prove of inestimable assistance in the standardization of clinical laboratory technic and personnel. As soon as one educational institution should have demonstrated the value of such an undertaking, others would surely follow. The results of such work would, in a very few years, definitely improve the professional character of the medical technician and materially assist in raising the standard of the clinical laboratory.

DISCUSSION

Dr. Robert A. Kilduffe.—I think we all realize that this is a very important subject a very important paper. It is impossible for any one to run a laboratory of any size without technicians and a good technician is a pearl beyond price. In order that the supply shall meet the demand there must be some source from which the supply shall come and this source Dr. King has endeavored to furnish. I think that Dr. King has outlined a very excellent and thorough course and I am in entire accord with him except for one thing: I do not think it either necessary nor advisable that technicians shall be instructed in the interpretation of the examinations they may make or help to make.

The word technician implies that their training shall be mainly in things technical. I demand of a technician, first of all, that there shall be no stalling. If he does not know how to do what I tell him to do, I do not want him to try and work it up out of a book. I like a technician to know why he does what he does, because I believe that this information leads to better and more careful work.

I want him to know that there is a definite reason behind the making of a blood count, but I do not think it necessary that he shall be taught the elements of blood count interpretation. This, it appears to me, is impossible, because for the intelligent interpretation of laboratory results much more is required than a smattering of information. I think that if technicians are led to believe that these interpretations are largely rule of thumb matters, which, of course, is far from the truth, the next step is the self-graduation of that technician into what he thinks is a clinical pathologist.

I think technicians should be trained to observe. I believe that a properly written report of a sputum examination, for example, should include some statement as to the gross characteristics and appearance of the specimen. I believe such observations properly constitute a part of the examination of the specimen.

I am opposed, however, to endeavoring to explain to technicians the interpretation of laboratory findings. I do not believe that it can be done in a short time, without a thorough preliminary training in a variety of subjects. I think that the main difference between a technician and a pathologist is the ability to interpret in terms of the patient and his condition the laboratory findings, and for this much more than mere technical training is required.

Dr. A. H. Sanford.—I believe that Doctor King's paper is worthy of further consideration and attention by this Society, perhaps next year. It is in many ways an important and as vital a subject as the standardization of laboratories. Technicians are part of the working material, part of the equipment. The University of Minnesota offers a course in medical technology leading to a B.S. degree. I have had girls who have had this course, and they were very efficient. Some others may not be so good. Some of the girls may get interested in medicine and later, as physicians, become clinical pathologists. Many of the young women applying to me for positions are school teachers, and they

often make good technicians. Another group is made up of registered nurses; they have to start back at the bottom, and occasionally they are willing to make the financial sacrifice. Still another group includes the bright high school girls, dexterous and willing to work. They are limited on account of lack of education, but they constitute a valuable and necessary part of a laboratory staff.

The "experienced technician" presents another story; she keeps flitting from tree to tree, with an increase in salary for every flit. The really good technician is a "pearl without price," and is often hard to keep. Medical technology as a profession offers a real field of endeavor for the educated woman. If she loves the work she will get a remuneration that is fair, and as a life work she will usually like it better than teaching.

Dr. A. S. Brumbaugh.—Doubtless you are all familiar with the psychologic tests for mental efficiency now commonly used by educators, and those who undertake to teach technicians should examine their mental caliber and perhaps give some test of that character. Those who do not measure up fairly well should be dissuaded from taking up this line of work.

Dr. Robert A. Keilty.—If the girls spend five or six years at college they are not going to do the ordinary routine at a low salary. I prefer to train them myself in my own way.

Dr. Walter E. King (closing).—1. Concerning the question as to whether the course which has been suggested is unnecessarily long or represents training which the average technician does not need, it seems to me there can be no argument. I cannot conceive of the idea of withholding knowledge from any student in any line of endeavor. The worthwhile student will seek the light and obtain knowledge regardless of obstacles. The technician's work involves such a diversity of knowledge and such a variety of manipulations and at the same time is so frequently being augmented by new methods that the student can scarcely reach the end.

2. The question as to the salaries of technicians can be answered without difficulty. The salary which the average well trained technician can command justifies a course of college training such as has been suggested. The salary of the technician compares favorably with the salary which is paid the average teacher.

3. It is recognized that there is chance for argument relative to the wisdom of teaching the interpretation of clinical laboratory findings to the student technician. It must be admitted that there is, at present, a tendency on the part of the hospitals to carry on the work of the clinical laboratory without the services of a medical director of the laboratory, depending entirely upon the findings and interpretation of findings by the lay technician. This imposes a responsibiity upon the lay technician for which he is totally untrained as he possesses no knowledge of clinical conditions. This condition of affairs is unjust to the patient and to the medical profession. I hope that I may be understood in attempting to express my opinion regarding the teaching of the interpretation of findings. I do not think that the lay technician should *interpret findings and sign reports.* The point which I do make, however, and which is clear to me is that for the successful teaching of the technician some stress must be laid upon the interpretation of findings and the application of various findings to clinical conditions. Such a course enables the instructor to fascinate the student in the entire work, to secure the undivided interest of the student and to turn out a much better finished product. Exhaustive study of laboratory interpretations is not necessary. It should be carried only as far as is necessary to hold the interest of the student and to satisfy him. Moreover, this procedure enables the student to appreciate the importance of his work and impresses him with the necessity of the most careful and painstaking effort. It seems to me that this idea of keeping the technician in the dark as to the significance of his findings accounts for the fact that many of these workers remain nothing more than routine drudges upon whose work no dependence can be placed. The lay technician should not be given the responsibility of reporting results and interpreting these results to the clinician. On the other hand, for teaching purposes and in order to develop the student into a highly trained technical assistant, such information should be freely given.

In conclusion, please allow me to state that in presenting these suggestions relative to the problem of supplying the proper course of instruction for laboratory technicians, my ideas have been the result of earnest consideration and intense interest.

FIELD OBSERVATIONS IN SCARLET FEVER*

By F. M. Huntoon, M.D., John A. Murphy, M.D., and S. H. Craig, M.D.
Glenolden, Pa.

THE work reported here was undertaken with the plan of testing different scarlet fever streptococcus toxins on the same persons, of studying its keeping quantities, and of checking the results of immunizing treatment as regards the elimination of the skin test.

The accompanying charts show the results obtained. In reading the results of Dick tests and retests, all reactions were measured in centimeters, and an area of redness of one centimeter was adopted as the minimum positive reaction. Intensity of redness was not included in the standard set for primary tests, but was noted in retests.

Heated toxin controls were used in the early part of the work. They were discontinued for the reason that only a little over one per cent of pseudoreactions occurred.

Table I is a composite chart confirmative of similar work done by other observers. Its most interesting point is that practically ten per cent of those giving a history of previous scarlet fever had positive reactions to the scarlet fever streptococcus toxin.

TABLE I
DICK TEST RESULTS

AGES	NO. TESTED	% POSITIVE
All Ages	2796	28.7
Age 1-10	474	47.4
Age 11-20	1717	26.0
Age 21-30	436	25.4
Age 31-40	151	8.6
Age 41-50	18	33.3
HISTORY OF SCARLET FEVER IN ABOVE GROUP		
All Ages	139	9.3

Two toxins from different sources were used, and some cross reactions were noted, in that a patient would be positive to one toxin and negative to the other. These cross reactions were recorded against both toxins, there being no constancy observed for any one toxin.

Table II shows a comparison between two toxins similarly prepared and is a composite chart.

While there is a difference in favor of toxin *A*, it must not be accepted that toxin *A* will consistently outpoint toxin *B*. The Drs. Dick[1] have recently pointed out the variation in the toxin production, between strains of scarlet

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From the Mulford Biological Laboratories.

fever streptococcus, stating the toxin production of any one strain is fairly constant. Our experience has been that two toxins, similarly prepared, will show some difference in percentage results, in favor of either toxin, in different groups tested.

TABLE II

DICK TEST

COMPARISON OF TWO TOXINS ON SAME INDIVIDUALS

(Both toxins freshly diluted unpreserved)

TOXINS	NO. TESTED, AGES 1-20	% POSITIVE
A. Hyg. Lab. Control Toxin	685	36.3
B. 1050 Toxin		31.2

(St. V.—G.M.—Mor.)

Tables III-*A* and III-*B* are concerned with the keeping qualities of scarlet fever streptococcus toxin diluted for the Dick test. The effect of preservative, temperature, age and method of preparation were studied. The groups tested are too small to aid in a definite conclusion and only serve as indicators.

TABLE III-*A*

KEEPING QUALITIES OF SCARLET FEVER STREPTOCOCCUS TOXIN DILUTED FOR THE DICK TEST

TESTS ON SAME INDIVIDUALS		
No. Tested	Freshly diluted unpreserved Hyg. Lab. Control Toxin	73708 Toxin Unpreserved 14 days at 37° C.
20	Positive 15%	Positive 15%
19	Same as above Positive 21%	Same—Preserved 0.5% Phenol 14 days at 37° C. Positive 10%
21	Same as above Positive 19%	Same—Preserved 0.5% Phenol Ice Box 14 days Positive 23%

(G.M.)

TABLE III-*B*

KEEPING QUALITIES OF SCARLET FEVER STREPTOCOCCUS TOXIN DILUTED FOR THE DICK TEST

No. Tested		
61	73708 Toxin Unpreserved Freshly Diluted % Positive 29	73708 Toxin Unpreserved Aged 30 Days % Positive 18 (St.V.)
46	73708 Toxin 0.5 Phenol Aged 30 Days % Positive 15	Same as above % Positive 50 (St.V.)
20	63 Toxin Purified Unpreserved Aged 50 Days % Positive 50	Hyg. Lab. Toxin Unpreserved Freshly Diluted % Positive 50 (U.P.)
157	63 Toxin Purified Unpreserved Aged 56 Days % Positive 19	Same as above % Positive 18 (T.)

Based on the results obtained, the indications are that:

1. A freshly diluted unpreserved toxin and an unpreserved toxin, kept at 37° C. for fourteen days are comparable.

2. The addition of preservative, under the same condition, is harmful to the toxin.

3. The addition of preservative, under cold storage conditions, is not harmful.

4. The same toxin unpreserved, freshly diluted and aged thirty days, shows an advantage for the freshly diluted toxin.

5. The same toxin aged 30 days, preserved against unpreserved, shows an advantage for the unpreserved toxin.

6, 7. A purified, unpreserved practically two-month-old toxin is comparable to an unpreserved freshly diluted toxin. This indication is supported by Table IV-*A*.

All our work in immunization was done by employing a series of three doses, 125, 250 and 500 skin test dose as originally recommended by Zingher.[2] We have never had with this dosage a severe, general or local reaction. Other workers, it is true, are employing much larger dosage, with an increase in the number and intensity of reactions produced.

It is questionable whether the use of the increased dosage is advisable, on account of the psychologic effect produced by severe reactions. This method, to be useful, must be applied as a public health measure, and in most communities, such severe reactions will block any general use of the method.

Table IV-*A*

Immunization Results at Varying Periods

		125-250-500 skin test doses	
Period	No. Tested	63 Toxin Unpreserved Aged 56 Days % Negative	Hyg. Lab. Toxin Unpreserved Freshly Diluted % Negative
20 Wks.	13	69	61.5 (T.)
16 Wks.	59	*Same as above % Negative 90	Same as above % Negative 90 (Mor.)
8 Wks.	20	Same as above % Negative 80	Same as above % Negative 80 (Mor.)
9 Wks.	45	73708 Toxin, Purified, Unpreserved Freshly Diluted % Negative 97.7	Same as above % Negative 97.7 (S.)

*250-500-1000 Skin Test Dose.

In this table a purified, unpreserved aged toxin and a purified unpreserved freshly diluted toxin gave consistent results against the control toxin. With the doses recorded here, there is no outstanding difference between them.

The immunization results shown compare favorably with other workers. Nesbit[3] in a series of 328 retests reported 65 per cent negative. In a personal

communication, Dr. Nesbit stated he would report later on two more series, using 2000 and 4000 skin test doses as the final dose.

The table indicated a higher percentage of negatives in the early weeks following immunization.

Reports from the Pennsylvania Soldier's Orphans School, Scotland, Pa., and the Pennsylvania Training School at Morganza, Pa., to the effect that three epidemics of scarlet fever had abruptly ended following the first immunizing dose given to the positive contacts, led us to check the time of disappearance of the positive reaction after immunization.

Tables IV-*B* and IV-*C* show the results.

TABLE IV-*B*
IMMUNIZATION RESULTS AT VARYING PERIODS
Retests with Two Toxins

	3 doses completed		2 doses completed	1 dose completed
	4 Wks.	12 days	5 days	4 days
No. Tested	72	37	8	4
% Negative	59	81	38	50

(Mor.)

TABLE IV-*C*
IMMUNIZATION RESULTS AT VARYING PERIODS
(125-250-500 Skin Test Doses)
Retests with Two Toxins

Interval after final dose	1 Wk.	2 Wks.	3 Wks.	4 Wks.	5 Wks.
No. Tested	6	24	36	32	41
% Negative	50	58.3	52.7	43.7	43.9

(U. P.)

The above results indicate that following the immunization a fairly large percentage show an early disappearance of the positive reaction.

In general, those still showing reaction show a diminution in intensity. There are some exceptions to this rule.

Furthermore, some individuals showing an early disappearance of the reaction again become positive within a few weeks.

The fact that in three instances in institutions epidemics have stopped short after the first immunizing injection (and this is supported by personal communications of instances in private practice) taken in conjunction with the early disappearance of the skin reaction in many individuals, indicates that the use of the toxin injections as a valuable emergency measure, regardless of the ultimate result in producing a permanent immunity.

Time only can decide the question of the permanent immunity produced.

Table V shows that a toxin prepared with and without preservative and aged eleven weeks has practically the same immunizing value.

TABLE V
IMMUNIZATION RESULTS WITH A PRESERVED AND UNPRESERVED TOXIN AFTER 11 WEEKS

	RETESTS WITH TWO TOXINS	
	73708 Preserved	73708 Unpreserved
No. Retested	19	18
% Negative	48	45

(C.B.)

TABLE VI

RETESTS ON A PREVIOUSLY NEGATIVE GROUP

	73708 Toxin Unpreserved, Freshly Diluted	Hyg. Lab. Toxin Unpreserved, Freshly Diluted
No. Retested	127	127
No. Negative	122	122
% Changed	4% Positive	4% Positive

(C.R.)

Table VI is of interest as it shows that 4 per cent of a previously negative group were positive when retested three months after the primary test. The two toxins used were the same in both tests, controlled with heated toxins in the first test and uncontrolled in the second.

SUMMARY

1. Phenol (0.5 per cent) as a preservative in scarlet fever streptococcus toxin diluted for the Dick test has little or no deteriorating effect when the material is kept at ice box temperature.

2. Preservative plus temperature of 37° C. causes a fairly rapid deterioration.

3. A temperature of 37° C. alone causes no deterioration.

4. Purified toxin (salt and acetic acid method) seems to be more stable than unpurified toxin.

5. The injection of immunizing doses of toxin apparently induces a prompt resistance to infection when used in the presence of an epidemic of scarlet fever.

6. A very early modification of the skin test is produced in many individuals by such immunizations.

REFERENCES

[1]Jour. Am. Med. Assn., March 14, 1925, lxxxiv, No. 11.
[2]Proc. of Soc. for Exper. Bio. and Med., May, 1924, xxi, No. 8.
[3]Nesbit: Jour. Am. Med. Assn., March 14, 1925, lxxxiv, No. 11.

DISCUSSION

Dr. John A. Kolmer.—Dr. Murphy is to be complimented and thanked for summarizing his work on this interesting subject in so clear and instructive a manner. If 500 or more skin doses of toxin may produce a scarlet-fever-like reaction when given for the first dose, is it possible to immunize with a toxin-antitoxin mixture more safely? Has Dr. Murphy any experiments to show that dissociation occurs after absorption? I doubt if the method would be objectionable from the standpoint of serum sensitization if a method could be developed along the lines of the T-A mixtures for active immunization against diphtheria.

I should like to ask Dr. Murphy how the antitoxic and antibacterial serums compare in their influence upon the streptococcus complications of scarlet fever?

Has Dr. Murphy any data to contribute upon the results of Dick tests in scarlet fever convalescents?

Dr. Frank M. Huntoon.—Our work has been done with comparatively small doses for the reason that we were afraid. Other workers not limited by lack of courage and other considerations have used very much larger doses. You can produce a negative skin test in practically every individual showing positive if you give enough material but by doing

this you may produce a very severe reaction. You are going to limit its value as a prophylactic. On the other hand, the commercial houses would not dare to put out some of the doses employed. We had a letter from a doctor in Buffalo who insisted that we supply him with stronger material. In injecting five hundred students for prophylaxis, one student showed all the symptoms of scarlet fever; four or five students reported for the second dose and none came for the third dose. That is the answer to the question of large doses. Large doses must be worked up to gradually if the immunization is to last a great length of time. After even one injection you get a considerable number whose skin tests become negative immediately. Some become negative, then positive, then negative again. What is the explanation of this? The skin test dose is dependent on two different factors; the first is sensitiveness of the skin; second, the presence of antitoxin in the blood. If we assume that the first skin test dose desensitizes the skin and then further assume that after a certain period, three, four, or five weeks, or longer, antitoxin appears in the blood, then we have the explanation for this very peculiar effect. If it is true that by desensitizing these people you can render them resistant to scarlet fever, that is a very important point.

Dr. Herman Sharlit.—I would like to ask if any observations have been made with reference to these immunization tests; whether there has been any effect noted on the kidneys of the people tested.

Dr. E. Clarence Rice, Jr.—We have been doing some work in conjunction with the public health service on immunization. We have finally given it up inasmuch as the dose of toxin was evidently too small and complete immunity had not been conferred, although the parents of some of the children injected considered them as protected for life, a false sense of security. I see no reason for not producing immunity, provided a sufficiently large dose of toxin can be used. One thing we have noted is that nurses who have been on duty in the scarlet fever ward and give no history of scarlet fever showed a smaller percentage of positive Dick tests than those just entering the hospital for training. It seems quite likely that scarlet fever may occur without a rash. Dr. Dochez feels that the unconcentrated serum should be used in preference to the concentrated, inasmuch as some of the bactericidal properties are lost in concentration. It is my opinion that the advantages of the unconcentrated serum are more than offset by the increased likelihood of serum sickness.

Dr. H. A. Heise.—We have given many injections of scarlatinal toxin with no bad results. A neighboring doctor gave his children the first dose with no noticeable effect. The second dose also appeared to give no reaction, but several days later the child complained of poor vision. Two weeks after the injection the eyes were examined and reported as right 15/100 and left 20/100. A reexamination in two weeks revealed practically normal vision.

Dr. David H. Bergey.—I was very much interested in this paper. It seems to me that if we could find some way of checking up the immunity and the amount of antibodies circulating in the blood of persons giving a positive reaction we would help this matter very much. If we could take the persons with the negative reactions, try various amounts of their serum and see what amount of serum would neutralize its toxin, we would obtain useful information.

Dr. John A. Murphy (closing).—In reference to the effect on the kidneys of the scarlatinal toxin in immunization, with the small primary doses I have no personal knowledge of any kidney complications. We had a report from one physician who said that he had given 500 skin test doses and that the patient had considerable swelling of the face and neck, the puffy condition of nephritis but no examination of the urine was made. Another physician using 500 skin test dose as the first dose, reported three of the five patients showed a trace of albumin in twenty-four hours and two of the three cleared after forty-eight hours. This would indicate that large primary doses may cause a mild transitory nephritis. Reactions, either local or general have been noted mostly after the first dose and seldom after subsequent doses.

Those individuals that show a rather intense reaction to the Dick test are rather hard to immunize; it is difficult to eradicate the positive reaction. Such individuals very probably have a high degree of susceptibility and will require a large total number of skin test doses to result in a negative Dick test.

The eye condition reported here was probably due to a mild nephritis. Dr. Kolmer's question on the use of a toxin-antitoxin mixture for immunization for scarlet fever, I cannot answer other than to say that it would probably protect in some patients, and in some there might be disassociation of the toxin and the antitoxin. Adults are more likely to show marked reactions to injections of the toxin than children.

As to the merits of the administration of the antitoxin in treating scarlet fever, it undoubtedly has beneficial effect.

THE BIOCHEMIC AFFINITY OF VARIOUS METALS AND METALLOIDS FOR BACTERIA AND ORGANIC TISSUES*

By Jay Frank Schamberg, M.D., and Herman Brown, B.Sc., Philadelphia, Pa.

THE present study was undertaken with the view of determining primarily the combining power of cancer tissue and of certain bacteria with various metals and metalloids. It was hoped that, if specific affinities could be demonstrated, the resulting data might furnish us with "leads" that would serve as a starting point for chemotherapeutic investigations. This assumption was based upon the dictum of Ehrlich that "Corpora non agunt nisi fixata." If certain chemical compounds exhibited a special combining power with particular bacteria or cells, normal or pathologic, in vitro, one would be encouraged to determine whether the administration of such compounds in vivo would manifest similar affinities.

The results of our studies in this direction were, on the whole, disappointing in that they tended to indicate that the combining power of the substances investigated were general rather than specific. Nevertheless the information derived possesses certain elements of interest that may possibly be of value along other lines of research. This prompts us to publish them at this time. The method which we pursued was to allow the salts of various chemical elements in solution to come in contact under standardized conditions with organs or bacteria and later analyzing the organic matter for the element adsorbed. We were particularly interested in the affinities for cancer tissues and tubercele bacilli.

This problem has been attacked from other viewpoints by a large number of investigators. The influence of various elements on the tubercle bacilli has been reviewed by DeWitt[1] and by Wells, DeWitt and Long.[2]

In 1915 Coenen and Schuleman[3] injected "eosin selenium" and various fluorescent derivatives into tumor mice and found that the living tumor cells have no special affinity for these dyes. Weil[4] analyzed tumor rats after injections of sodium iodide and found that the blood contained the highest amount of iodine, then, in order, the tumor and the liver. His results indicated, too,

*From the Research Institute of Cutaneous Medicine, Philadelphia.

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ADSORPTION OF ELEMENTS FROM INORGANIC SALTS BY VARIOUS SUBSTANCES

NO.	COMPOUND USED	ELEMENT DETERMINED	MG. OF ELEMENT ADSORBED PER GM. DRY SUBSTANCE											
			TUBERCLE BACILLI	STAPHYLOCOCCI	NORMAL CALF'S BRAIN	SHEEP'S LIVER	SHEEP'S HEART	CANCER (LIVER)	CANCER (BREAST)	CANCER (LIVER)	ATOMIC WT. OF ELEMENT	PERIODIC GROUP OF THE ELEMENT	SERIES IN THE PERIODIC SYSTEM	APPROXIMATE PH
1	Auric Chloride	Gold	175	144	163	107	116	180		179	197.2	1	11	4
2	Mercuric Nitrate	Mercury	170	107	115	103	130	86		92	200.6	2	11	3
3	Pot. Tartro-Bismuthate	Bismuth	84	58	94	124	78			72	209.0	5	11	9
4	Uranium Nitrate	Uranium	80	130	77	91	70	89		84	238.2	6	12	6
5	Lead Nitrate	Lead	61	89	88	71	57	99		89	207.2	4	11	4
6	Silver Nitrate	Silver	41	102	67	39	31	52		60	107.9	1	7	6
7	Vanadium Dichloride	Vanadium	35						5		51.0	5	4	3
8	Cupric Sulphate	Copper	28	24	23	13	11		5	16	63.6	1	5	7
9	Ferrous Ammonuim Sulphate	Iron	21		23	8	8	6		9	55.8	8	4	7
10	Nickel Nitrate	Nickel	17						4		58.7	8	4	5
11	Sodium Tungstate	Tungsten	15								184.0	6	10	10
12	Potassium Iodide	Iodine	11								126.9	7	7	8
13	Sodium Tellurate	Tellurium	10						2		127.5	6	7	12
14	Arsenic Pentoxide	Arsenic	9			29	22	24			75.0	5	5	1
15	Thorium Chloride	Thorium	9						4	4	232.2	4	12	7
16	Sodium Selenite	Selenium	8		7				2	5	79.2	6	5	12
17	Barium Chloride	Barium	6		13	5	4	8			137.4	2	8	6
18	Calcium Chloride	Calcium	10						4		40.1	2	4	4
19	Zinc Chloride	Zinc	4		6				2	3	65.4	2	5	4
20	Magnesium Sulphate	Magnesium	4		10					6	24.3	2	3	7
21	Sodium Silicate	Silicon	4								28.1	4	3	14
22	Cesium Nitrate	Cesium	3								132.8	1	8	10

that diazo dyes produced an intense coloration in the necrotic areas of the tumors. The tissues were but slightly stained.

Cramer[5] exposed, in vitro, cells of a transplantable mouse carcinoma to the action of equimolar solutions of sodium chloride and calcium chloride. Cells treated with the latter showed a distinct inhibitory action. Sugiura, Noyes and Falk[6] investigated the growth of Flexner-Jobling rat carcinoma after grafts had been immersed in solutions of various salts at various hydrogen-ion concentrations. They found that Ca strongly inhibits growth and that a P_H of 6.0 appears more harmful to the carcinoma than a P_H of 8.0. Sugiura and Benedict[7] studied the effect of 32 different salts, administered orally, on the growth of Flexner-Jobling rat carcinoma. Copper sulphate, arsenious oxide, potassium carbonate and calcium chloride showed a perceptible, though not marked, retarding influence upon the growth of the tumor. Copper sulphate was the most effective in this respect and appeared to have some immunizing action against the tumor. Analyses made by Robin[8] indicate that cancerous tissue fixes silica, lime and to a lesser degree magnesium.

Our work consisted in exposing the bacilli or finely ground normal or cancer tissue to the action of solutions of various salts, removing the excess of solution, washing and analyzing the residue for the adsorbed element that could not be removed by repeated washing. The results are presented in the table.

DISCUSSION

Eight series of experiments were carried out with tubercle bacilli, staphylococci, cancer of human breast, cancer of human liver (two experiments), sheep's heart, sheep's liver, and calf's brain.

Examination of the above table discloses the fact that gold and mercury lead the list in combining power with bacteria and tissues, with bismuth, uranium and lead following in sequential order.

We were at first rather encouraged that gold and mercury exhibited the greatest affinity (among the elements tried) for tubercle bacilli, as these two metals in one form or another have been long used in tuberculosis and are believed by some to exhibit an influence upon the disease. The suggestive value of the findings was, however, largely nullified when we discovered that these two metals also exhibit a high degree of affinity for the staphylococcus and for various organic tissues.

It will be noted that all of the substances at the top of the list have high atomic weights, but on the other hand thorium and tungsten, which have likewise high atomic weights, exhibit a low combining power. The substances at the head of the list fall in different "periodic groups of elements," so that the periodic grouping exhibits no influence upon the behavior in relation to proteins.

It is rather interesting to note, however, that four of the five elements that show the greatest combining power with organic material are in the eleventh series in the periodic system, and the fifth is in the twelfth. Whether or not this observation possesses any significance, further study alone can determine.

That the method employed by us gives reasonably accurate results is suggested by a comparative study of the experiments with two different specimens of cancer of the liver made at different times. In the first test 180 mg. of gold

was recovered and in the second 179 mg. The figures for the other elements, while a little wider apart, nevertheless indicate that the technic used is thoroughly reliable.

METHODS

The tubercle bacilli (human and bovine mixed) were washed free of glycerine, dried between filter paper, a ten-gram sample removed for drying to constant weight at 105° C., and the remainder weighed in five-gram lots into 50 c.c. centrifuge tubes. To each were then added 20 c.c. of a solution of such concentration that it contained 2 per cent of the element to be determined. The tubes were stoppered, thoroughly shaken, heated at 37° C. for three hours, and placed in the ice box overnight. The mixtures were then centrifuged and the supernatent liquid discarded. About 30 c.c. distilled water were added, and the whole mixed, centrifugalized, and the supernatant liquid discarded. This process was repeated seven more times except that, before discarding the sixth washing, tests were made to determine whether the wash water still contained any of the element to be determined. It was found that the sixth washing was generally negative. The bacilli were washed twice more, however, drained as completely as possible, and transferred quantitatively to various containers to be analyzed for the adsorbed elements.

The staphylococci were obtained dry. Two-gram lots were used and treated as above.

All the tissues were passed through a small meat chopper; ten grams were removed for the moisture determination; the remainder was weighed into centrifuge tubes, ten grams in each, and treated as above. All results were calculated to a dry basis.

For the determination of mercury,[9] bismuth, lead, copper, iron, nickel and tungsten, the organic matter was destroyed with concentrated sulphuric acid and potassium sulphate in the ordinary Kjeldahl manner. Mercury and bismuth were weighed as the sulphides; lead as the sulphate; copper precipitated as sulphide and ignited to the oxide; iron and tungsten as the oxides; and nickel as the dimethylglyoxime.

For the determination of gold, uranium, vanadium, tellurium, selenium, thorium, magnesium, zinc, and silicon, the organic matter was destroyed with aqua regia. Gold, selenium and tellurium were weighed as the metals; uranium, vanadium, thorium, magnesium and silicon as the oxides; and zinc as the pyrophosphate.

For the determination of silver, barium, calcium and cesium, the organic matter was destroyed by ashing. For the silver estimation the ash was leached with concentrated nitric acid; for barium and calcium, with dilute hydrochloric acid; and for cesium, with dilute sulphuric acid. Silver was weighed as the chloride; and barium and cesium, as the sulphates. Calcium was estimated by titrating the oxalate with standard permanganate.

For the arsenic the organic matter was destroyed on the water-bath with hydrochloric acid and potassium chlorate; the arsenic precipitated as the sulphide, dissolved, reprecipitated with magnesia mixture, and weighed as magnesium pyroarsenate.

ward to the development of the ideas which mark a new departure from the old conservative system.

We have seen that the metronome type of recorder has many advantages in accuracy, and disadvantages in the limited range, exposed mercury cups, and in some cases a spring tension for activating the pendulum, which is pivoted at the bottom.

From this departure we come to the clock type. In appearance these simpler forms have much the semblance of the common wall or grandfather's

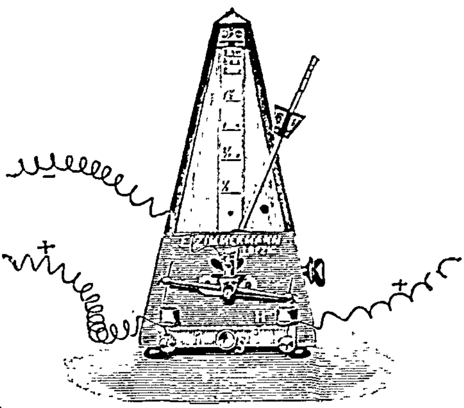

Fig. 1.—Metronome type, with exposed mercury cups, spring driven. Range from ⅕ to 3 seconds.

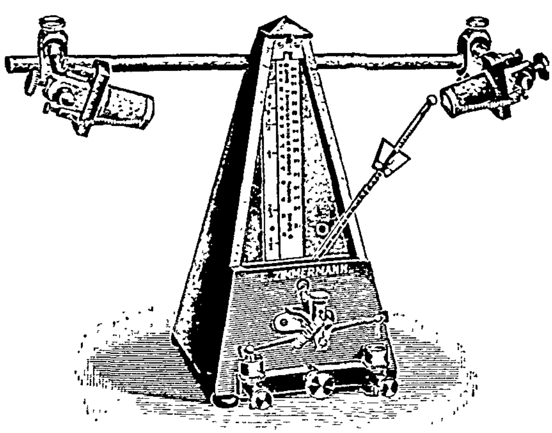

Fig. 2.—Metronome type (electrical) having exposed mercury cups. Range from ⅕ to 3 seconds. Electrically propelled.

clock. There are numerous models, but here we can refer briefly to some of the representative types only.

Fig. 3 represents well one of the more simple types which consists for the most part of a pendulum activated by a weight which pulls on the drive shaft, furnishing power to the ratchet which, through a pawl, activates the pendulum, the swinging of which tilts an arm. This arm is pivoted in the middle, and is connected to the pawl. The contacts are made similar to those of the metronome shown in Fig. 1.

Fig. 4 is the contact clock according to Bowditch-Baltzar. Through the

swinging of a carefully adjusted "second" pendulum the contact point is moved forward one notch, and by means of a moving strip of metal the current is broken. The contact points are pegs of metal, arranged in concentric circles, accurately spaced so as to record intervals of 1, 2, 3, 4, 5, 10, 15, 20, 30, and 60 seconds respectively. By making adjustment, the contact points can be made to so respond that the *break* stimulus can be given instead of the *make*. The workmanship on the rachet and pawl is such that the interval of time is very accurate, yet the mechanism of the clock is very simple. It will be noticed that there is still a close adherence to the pendulum type of recorder all the way through this series. Of still greater importance are the following combinations of the pendulum and electromagnet types. In these clocks the weight and spring are not used, yet the pendulum is retained.

Fig. 5 is a cut of the late Prof. Brodie's pendulum time-marking clock.[1] This clock consists of a half-second pendulum, the swing of which is maintained

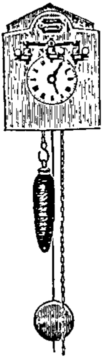

Fig. 3.—Pendulum type, with mercury cups. Weight, ratchet, and pawl activation.

Fig. 4.—Contact clock (Bowditch—Baltzar). Pendulum type activated by weight. Contact points are metal.

electromagnetically. The pendulum acts by means of a pawl upon a ratchet wheel, which has sixty teeth, and is moved forward one notch (tooth) for each double swing of the pendulum. As it moves, one of the teeth at the side of the wheel is made to depress a spring, and thus close the circuit of the writing signal. This gives a sharp tracing by the chronograph. On the same axis are other ratchet wheels, each having a different number of teeth, depending upon the time interval they are to give. In the clock as reported, there were wheels having 30, 6, 2, and 1, respectively. This would mean that the contacts could be made at intervals of one, two, ten, thirty seconds and minutes.

Fig. 6 shows the Harvard electric clock. This was reported by W. T. Porter,[2] and would come in this class. This is a portable type and is of the same general design. So far as the time interval is concerned, it delivers the same rates as does Fig. 5. In action, a pendulum moves a pawl, which each

second turns a wheel one-sixtieth of its circumference, making a contact which activates a magnet to keep the pendulum "Normal." On the rim of the wheel are contacts, which cause a magnet (signal) to record each one, two, ten, thirty, and sixty seconds as does the one illustrated in Fig. 5. The driving circuit is independent of the time circuit. The signal contact spring can easily be moved along the transverse bar, bringing it in contact with the wheel delivering the time interval desired. A leveling device is provided, which is necessary when one hopes to use it as a portable instrument. Leveling of this instrument is facilitated by the addition of a point on the base over which the pendulum is carefully balanced when at rest.

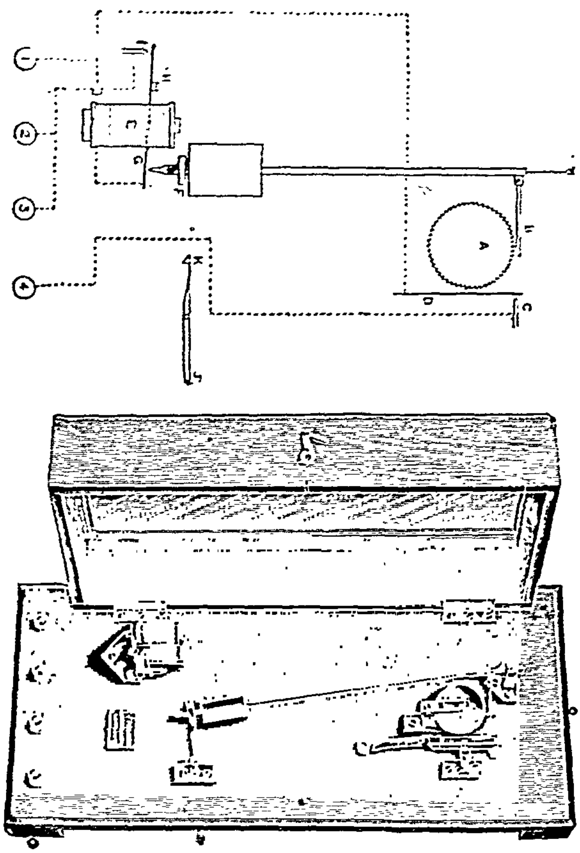

Fig. 5.—Pendulum time-marking clock.[1] Electrically activated. Contact points are metal. (T. G. Brodie.)

Through this entire series of pendulum type clocks we have observed several striking contrasts, both in application of the driving mechanism, and in the manner of making the contact. A combination of many of these (except the mercury-cup principle) with many refinements, will be found in the astronomical clock after Clemens Riefler,[3] Munich, Germany. (One of these clocks will be found in use at The Bureau of Standards, Washington, D. C.) It is claimed to be the most accurate clock in the world, the probable error lying somewhere between 0.01 to 0.02 of a second in twenty-four hours.

Some idea of its accuracy can be obtained from some illustration which brings to mind the linear error on a very fast drum. This of course will vary

according to the lag in the chronometer; the resistance in the lead-in wires, and several other factors (making difficult a very accurate measurement). Then we shall profit more, in our attempt to illustrate its accuracy, by disregarding these extrinsic factors, and deal with the clock itself.

If we were to picture a six-inch Harvard, "slow and fast combination" kymograph, revolving at a rate of once per second, and the recording instrument executing the contact made within the clock, without lag, the linear error would be slightly less than one-tenth micron. Another illustration might be

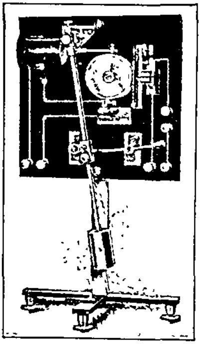

Fig. 6.—Harvard electric clock. Portable pendulum type. Electrically activated. Adjustable. (W. D. Porter.)

Fig. 6-*A*.—Astronomical clock. (Riefler.)

used. If the error recorded were superimposed upon an accurate scale, a high-power microscope would be necessary to detect it. By careful installation and proper adjustment it is possible to maintain this accuracy, constantly.

It is enclosed in an air-tight glass chamber to which are connected means for partially exhausting the air, thus changing the pressure within the clock to any desired amount. After the proper adjustments are made, pertaining to pressure, the clock requires little attention.

It is wound every half minute by a battery of dry cells (4.5 volts) the average life of which, for this purpose, is six months.

The pendulum is supported on agate planes, and operates a wheel having sixty teeth, one of which may be removed to indicate the beginning of the minute. A ruby jewel, bearing upon this wheel, operates an electric contact each second as the jewel passes over the teeth. This contact opens the circuit of a relay, which distributes the signals to the various laboratories, for operating chronographs.

It will be seen that the pendulum type of recorder is used in this most accurate instrument, and that the spring, the source of power, is kept at a constant tension by frequent windings. The effects of atmospheric changes are reduced to a minimum by the glass encasement, as are convection currents.

Further information may be obtained, pertaining to the construction and cuts, if reference be made to D.R.P. No. 100870, München, 1903.

A photographic reproduction is shown in Fig. 6-*A*.

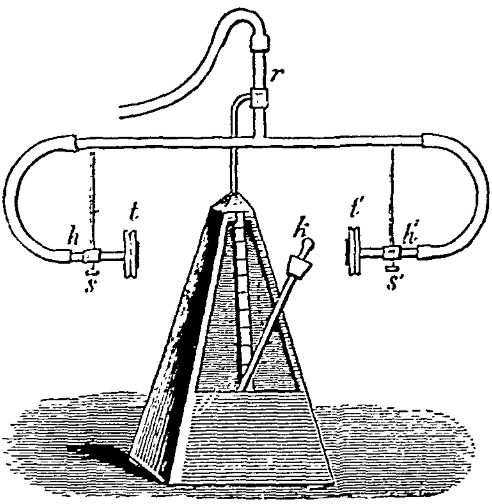

Fig. 7.—Transmission chronograph. Metronome type. Activated by spring. Impulse is transmitted through a closed system to recording instrument. (Klemensiewicz.)

Before leaving the pendulum type of recorder, we must refer to the transmission chronograph (Klemensiewicz). This is an air transmitting instrument, consisting of a metronome to which at its upper portion is connected a *C*-shaped pipe in such a way that the swinging of the pendulum strikes against tambours, one on each end of the curved pipe. By each movement of the pendulum the tambour is struck, causing a depression in the diaphragm. This in turn is transmitted through the closed system to the recording tambour. It will be noticed in Fig. 7, at the point *r* a tube (rubber) is connected which transmits the impulse to the recording apparatus. The pendulum is activated by a spring.

Departing from the pendulum type of recorder we come upon a class which marks a distinct innovation in time-recording apparatus. They are small and portable and more especially designed for individual work. Accuracy will be found in this class of instrument equal to that of the pendulum type. One of the simplest of this class will be found illustrated in Fig. 8. This recorder

was described by C. C. Lieb.[4] It consists of an Ingersoll watch to which is attached, instead of the second hand, a toothed, geared wheel which activates a writing lever. Time may be recorded in minutes alone; in minutes and seconds, or in minutes and five second intervals. The writing lever is made of straw; the writing point of celluloid.

Without doubt the most accurate and complete, small, portable chronometer on the market today will be found in the Jaquet graphic chronometer. It consists of an accurate clockwork with anchor escapement; two dials on one side, one of which totals seconds, and the other, the minutes of operation. A small lever placed beneath the box will set the instrument at *o* instantly. It is also arranged to give an electric signal through a magnet, being provided with binding posts on the front side. This contact for signaling the beginning

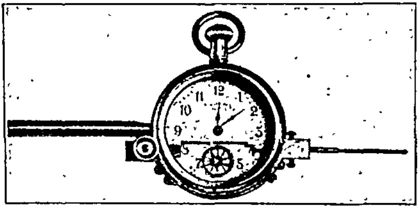

Fig. 8.—Time marker (Lieb), consisting of an Ingersoll watch to which is attached, instead of a second hand, a tooth-geared wheel. This wheel turns 1/60 of a complete revolution at each escapement of the anchor, activating the writing-point. It is adjustable to deliver different rates of time.

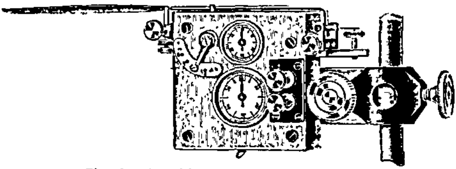

Fig. 9.—Graphic chronometer. (Jaquet.)

of the observation, is made with the lever which establishes the zero reading. It can also be made to record time at a distance. It will write in a vertical or horizontal position. It appears on the market in two executions, and is capable of delivering one-fifth, one, three, and six seconds. This class of instrument is the only type taking advantage of the anchor escapement principle. An illustration of this chronometer is given in Fig. 9.

Having considered well the advantages and disadvantages of all the different types of chronographs and time markers, and having worked with many of them personally, it appeared to me that instead of having individual time markers, which, by the way, are subjected to use by inexperienced workers principally (that is for student's laboratory work) and in a way are very satisfactory, but are apt to be out of order when most needed, it would be a remarkable advance, so far as handling a student's laboratory is concerned, to have a central clock, capable of taking care of the entire laboratory, delivering

to any or all tables therein the time interval most suited to the needs of each individual experiment. This led to the summing up of the needs of the average modern laboratory. In this summing up it was found that there were needs for the following: time in one second; seconds and minutes together (to facilitate counting time, and analyzing records) and then an increased rate to compare with the tuning fork of about one one-hundredth, or at least one-tenth of a second. This gives a range taking care of average experimentation, and the fast rate for measuring the phases of individual tracings on a fast drum. This refers to records of time. Then there is a great need for the automatic stimulating machine; something that will give stimulation through the inductorium at a constant rate and intensity; one that can be varied to suit the needs of the experiment without altering the time record. All this necessitates a somewhat complicated mechanism, simple to operate, and positive in execution. In fact, the percentage of error present with either without the other is so high that even the inexperienced experimenter will ask for explanations.

With these things in mind the University of Maryland chronograph[5] was produced. It has been in use in our laboratory, and without an exception has filled all the needs of our students' laboratory. Not only is it accurate in the rate of stimulation, and time recording, but constant in intensity, and simple to operate. An illustration of recording time in seconds and minutes, and at the same time stimulating the gastrocnemius muscle of a frog, is given in Fig. 10. It will be seen that the rate of stimulation was started at about one in three seconds, then slowly increased until it was being stimulated approximately twice per second. At about the center of the record as indicated thereon, the rate was slowly decreased until the original rate was established. The kymograph paper upon which this record was made is not the glazed surface variety, but regular Drug Bond wrapping paper. A celluloid point was used on the recording lever. At the time this record was made six other tables were in use. Table one was using minutes only; table two was using seconds and minutes and tables three, four, five, and six were using one-fifth of a second. This shows the possibility of any one table having at its service the time interval best suited to its needs. There will be a record shown illustrating the different rates possible at any one time.

Fig. 11 is shown to give some idea of the kind of record made when standard apparatus is used. The paper upon which this record was made is Drug Bond wrapping paper. The kymograph was a standard Harvard, which is driven by a spring. It will be seen that for fast records the rate is not constant, but in this record where the character of the signal is to be illustrated that does not interfere. The rates as indicated on the record, therefore, can be given only approximately, so far as the speed of the drum is concerned. The contact is made and maintained for one one-hundredth of a second. This throws the *make* and *break* so close together that even in a fast drum record, such as can be obtained with the Harvard "slow and fast combination" kymograph, it is not possible to distinguish the break. In many kinds of experimental work this is an advantage. It will be noticed in this record that all contacts are sharply registered on the record. The "minute" can be recorded

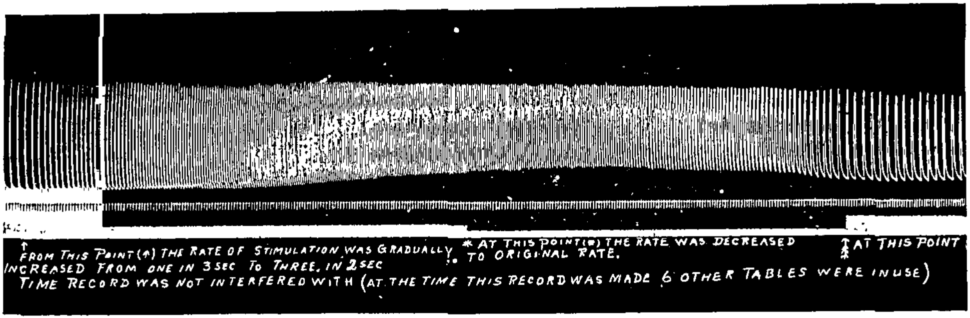

Fig. 10.—The broad band below the record at the first arrow (↑) shows the point at which the rate of stimulation was gradually increased from one in three seconds to three in two seconds. The asterisk (*) indicates the point at which the rate was gradually decreased to the original rate. The time record was not interfered with, nor were the instruments being used on the other six tables.

either by a break in the line, of a second duration, or by a sharp single line, depending upon the adjustment of the timer. These different methods of recording minutes will be found on the record as shown in Fig. 12.

Fig. 12 is a record showing the different time intervals that can be given at one time to any or all of twenty tables. The number "twenty" is mentioned because of the instrument being described, it having been made to care for a laboratory of twenty tables. It can be made by the insertion of a dif-

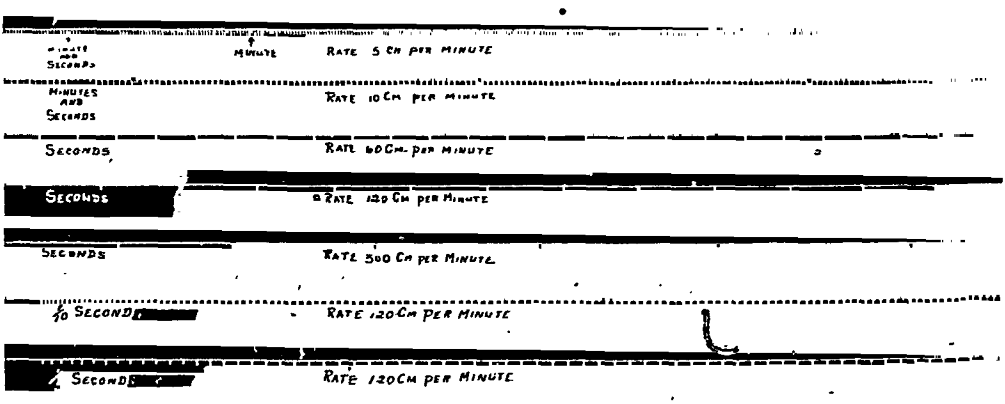

Fig. 11.—Showing how different time records will appear when kymograph is revolving at different rates. Also how seconds and minutes are recorded with one magnet.

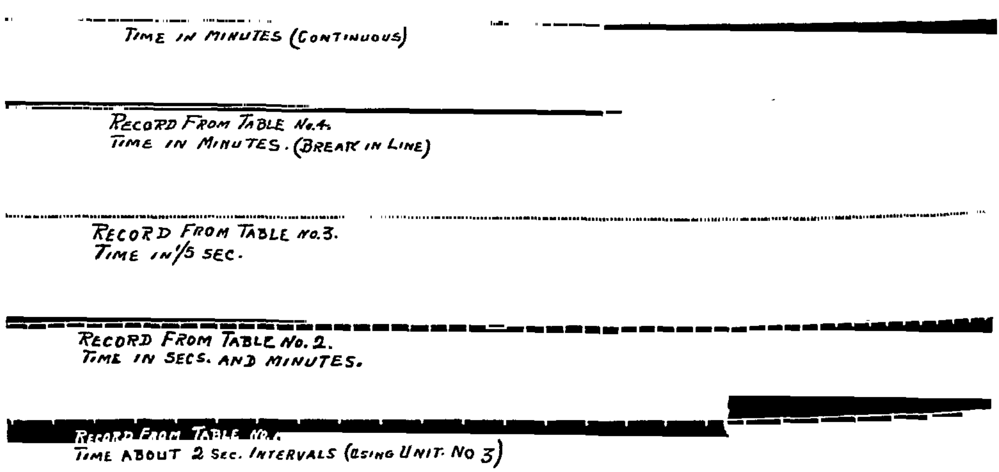

Fig. 12.—The top line shows the record made when minutes are being recorded and the timer is set to make a sharp contact. The second line illustrates how minutes can be recorded on a very fast drum when the timer is set to cause a break in the line lasting one second. The third line shows 1/5 second as recorded with a standard Harvard Magnet. The fourth line shows minutes and seconds on a fast drum. The bottom line is shown to illustrate the use of unit No. 3 for giving time at intermediate rates.

ferent commutator, to take care of even one hundred or more. That is, it can be made to care for the needs of the number of tables mentioned without having any two tables operate from the same line at the same time. The first record at the top of Fig. 12 shows how minutes may be recorded on a very

fast drum when the commutator is adjusted to deliver a sharp contact. The second record (Fig. 12) shows the commutator adjusted to give a contact of one second duration. The third shows how one-fifth of a second will be recorded, and the fourth, seconds and minutes, with the commutator of the minutes unit set to make a break in the line, lasting for exactly one second. It should be mentioned here that while the magnet is being held down, during that second, recording minutes, no other table is being interfered with. The instrument is so made and adjusted that an entirely independent current is operating the minute signal, while the second signals are continuing on their own circuit. The fifth record (bottom) shows the possibilities of shunting into the time recording service, the unit for stimulation which makes it possible to get time in either half seconds or two seconds, or a fraction thereof. The record here shown is time at a rate of approximately two second intervals. This is adjustable, and is the one unit that can be fitted to the needs of special work. This illustrates the use of the chronograph, so far as the time recording possibilities are concerned, showing how the entire instrument can be applied

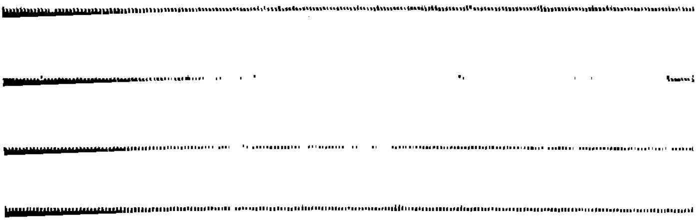

Fig 13.—Showing how the chronograph records the different time intervals when all magnets are started on a vertical plane. This record was made by connecting four tables together in such a way that their magnets would record on one drum.

to the recording of time or to both time and stimulation, as the experiment requires. The records as given in Fig. 12 were made simultaneously, the magnets being connected to leads from different tables as indicated on the record.

Fig. 13 illustrates how the records would appear if the tables were all writing upon one drum simultaneously, provided the points of the magnets were all started upon a vertical line. It will be seen that the record at the bottom records minutes first; three seconds later, the second magnet records, and three seconds later, the third and so on. By the time the last of the twenty tables records minutes the first will be within three seconds of recording minutes again. No two tables record minutes simultaneously. This allows some little time for the chemical reaction in the battery to take place and insures positive execution.

It will be seen from the records shown that there is not a trace of interference, one table with the other. While all tables receive their current at a definite rate, at no time are there two receiving impulses from the chronograph. This involves a perfectly timed commutator, a compound circuit, and

a constant "input." These important parts are believed to be well represented in this instrument.

Fig. 14 is a perspective of the University of Maryland chronograph. The rheostat (*R*) is a Beck Brothers 750 Ohm 0.6 Amp., and governs the input to the motor (*M'*) which is a Hamilton Beach Home type, rated ¼ h.p. *AC* or *DC*, 3000 r.p.m., and drives the machinery through a spiral gear *S'*. This

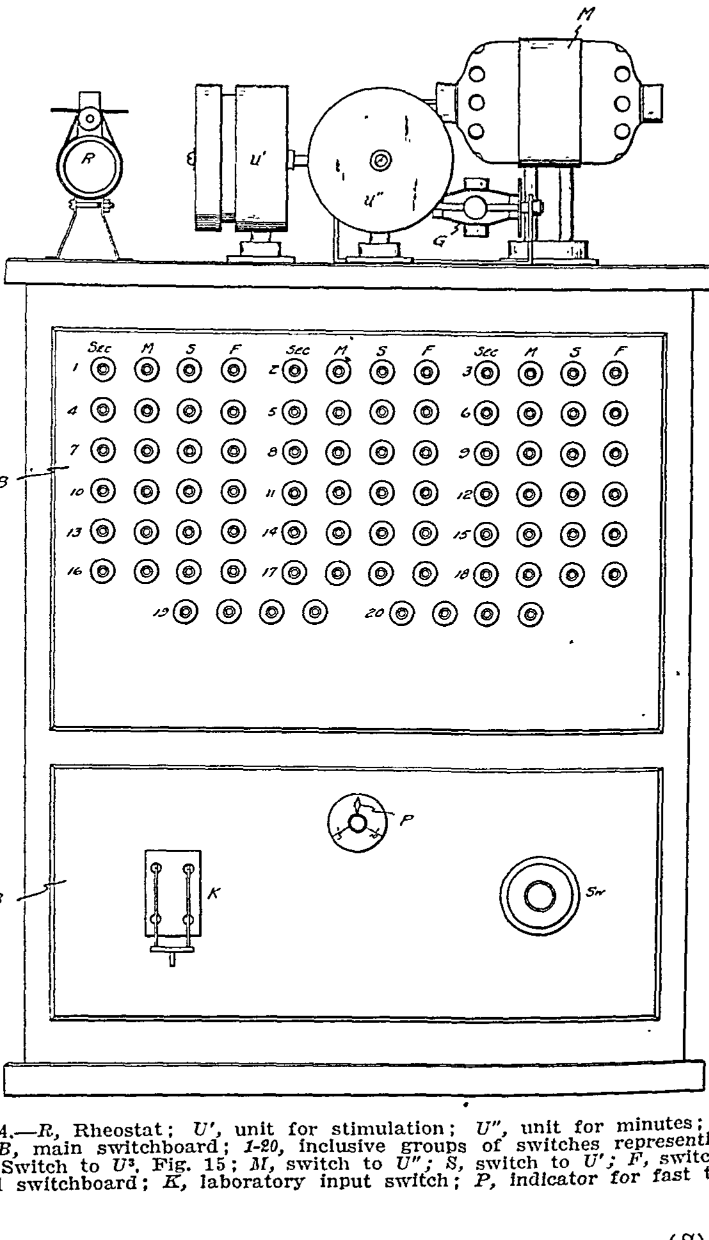

Fig. 14.—*R*, Rheostat; *U'*, unit for stimulation; *U''*, unit for minutes; *G*, governor; *M'*, motor; *B*, main switchboard; *1-20*, inclusive groups of switches representing laboratory tables; *Sec.* Switch to U^3, Fig. 15; *M*, switch to *U''*; *S*, switch to *U'*; *F*, switch to indicator *P*; *B'*, small switchboard; *K*, laboratory input switch; *P*, indicator for fast time; *Sw*, 110 volt switch.

worm drive engages with a 32 pitch (diametral) spur gear (*S*) having 80 teeth (Fig. 15). This gear is fastened to the driving shaft, "pressed on," and becomes the driving shaft proper, common to all units (*D*) (Fig. 15).

In Fig. 14 *U'* is the unit wherein the contacts are made which give stimu-

lation at intervals varying from half seconds to three seconds; U'' the unit which makes the contacts for minutes. There is another unit, which is not shown in this perspective, lying between U'' and the motor. This unit is the "second unit" and can be seen in Fig. 15.

The governor (G) is a "three ball breaking type," and engages with the drive shaft (D) through a worm and spur gear (w) (Fig. 15). The efficiency of this device is marvelous.

The switchboard B (Fig. 14) is fitted in, and becomes the upper panel of the front of the cabinet. On it are mounted the switches in groups of four, twenty in number, making a total of eighty. The small numbers appearing before and on the left side of each group of four switches, represent the numbers of the tables to which the wires of that particular group run. The lettering over the vertical rows of switches represents the execution. For example, *Sec.* for seconds, *M*, for minutes, *S*, for stimulation and *F* for fast time. In order to have fast time at either one-tenth or one-fifth of a second it is necessary to turn indicator P (Fig. 14) to the point desired, and then pull switch marked F. If fast time is not desired, it is not necessary to readjust the indicator P but simply use switch F.

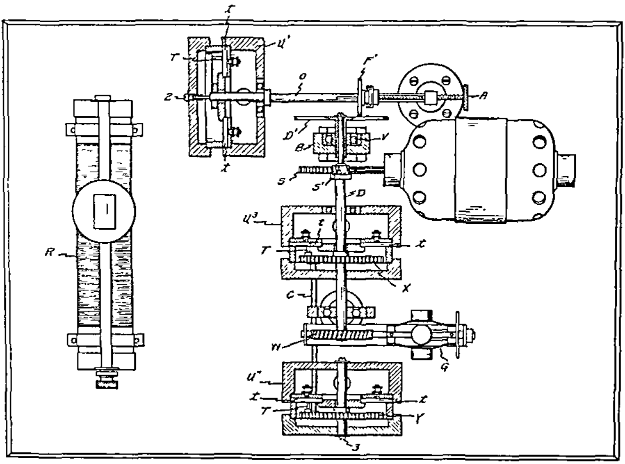

Fig. 15.—*R*, Rheostat; U', unit for stimulation; U'', unit for minutes; U^3, unit for seconds; *2*, ground-wire to unit U'; *T*, brush; *t*, contact; *O*, drive shaft; F', friction wheel; D', friction disc; *A*, screw set; *B*, thrust bearing unit; *V*, new departure ball-bearing No. 1200; *S*, spur gear 80 cogs; S', spiral gear; *D*, propeller shaft; *X*, gear wheel (one cog); *Y*, gear wheel 80 cogs; *W*, spur gear to governor; *G*, governor; *3*, ground-wire (common to unite U'' and U^3).

Another switchboard B' forming the lower panel in the front of the cabinet, contains the double knife switch K which connects the laboratory tables to the instrument (chronograph). It is in the circuit on the "ground" side. Indicator P is mounted also on this panel, the explanation of which was

given in the preceding paragraph. The diagram *P* (Fig. 20) explains the wiring. The core of this indicator is an insulated brass-bakelite commutator which when indicator *P* (Fig. 14) is turned to one-fifth of a second serves as a common conductor for five of the twenty contacts, and is shunted through switch *F* (Fig. 14) to the table desired. The same principle applies to the

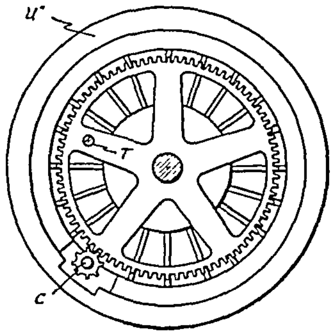

Fig 16

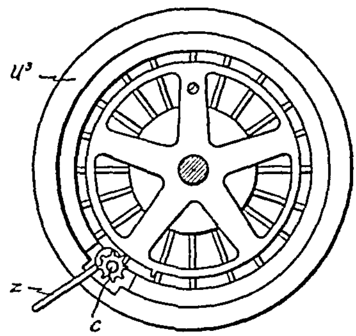

Fig 17

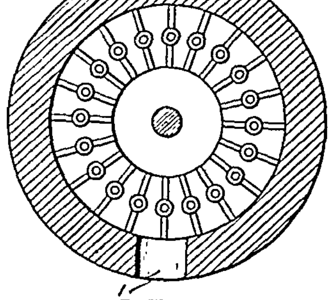

Fig 18

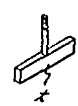

Fig 19

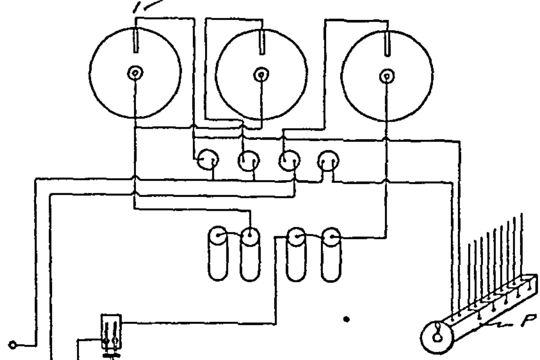

Fig 20

Fig. 16.—*U''*, case of unit for minute contacts; *T*, brush; *C*, cam shaft.
Fig. 17.—*U³*, case of unit for second contact; *Z*, timer; *C*, cam shaft.
Fig. 18.—Reverse side of units *U''*, *U'*, and *U³*; *1*, hole for support.
Fig. 19.—*T*, contact (actual size).
Fig. 20.—Wiring diagram showing all three units; one group of switches; four dry cells (Red Seal); laboratory input switch; three binding posts (on laboratory tables); fast time unit (*P*).

one-tenth of a second except that in this case ten of the contacts find the commutator a common conductor. It will be seen here, that there can be no division of these two time intervals. The entire mechanism is either delivering all one-fifth or all one-tenth. It is easily rearranged, in that the core is responsible

for this execution. The driving switch 110 V. *Sw* completes the trio of mountings. Through this switch the AC or DC current is supplied to the motor through the rheostat (*R*).

Fig. 15 is a cross section of the contact units, bearings, friction transmission, and gearing. The unit, *U′* is responsible for stimulation, the rate of which is governed by the position of the fiber wheel *F′*. This wheel is adjusted to suit the needs, by use of the "screw set" *A*. Having been set at the point best suited for the experiment, the disc *D*, by means of another "screw set" not shown on this cut, but at the base of, and under the bearing unit *B*, is brought into contact with fiber wheel *F′*. This starts the propeller shaft *O*, to which is fastened the contact brush *T*, (this brush is made of 50-50 half hard brass) causing contacts to be made on each of twenty inlaid-contacts *t*, also *t* (Fig. 19). This unit can be thrown out of service at will. It will be noticed that the disc, and its thrust unit is firmly connected to the bearing *V*, and that the entire unit slides over a key in the propeller shaft *D*. This keeps the wheels at right angles to each other and the disc in a vertical position at all times.

The unit, U^3, gives the contacts for seconds. Its construction is similar to that of *U′* except that the brush (*T*) is attached to the gear wheel (*X*). Unit *U″* delivers minutes and is driven through a cam shaft from unit U^3. In each complete revolution of the wheel *X* of unit U^3 a cog engages with the cam turning the gear wheel *Y* of unit *U″* one-eightieth of its circumference. This causes the brush to rest for one second on each contact as it revolves, providing the timer *Z* (Fig. 17) is so adjusted. It can be made to rest on either side of the contact. In this case it passes over without interruption and makes the contact in one one-hundredth of a second. This gives a sharp signal by the chronometer. Otherwise it gives a break in the line of one second duration.

Fig. 16 illustrates the relation of the cam (*C*) to the contact surface, the position of the brush, the actual size of the unit, and the "open fan arrangement" of the contacts. This is common to all units.

Fig. 17 gives the inside appearance of the unit U^3 showing the timer, cam, and brush.

Fig. 18 gives the reverse side of the contact unit, showing how the connections are made to the contacts, also the hole into which the unit supports are inserted, *1*. These contacts should be soldered to avoid loose connections. The support is a hollow pipe through which the leads disappear to the cabinet and switchboard.

Fig. 19 shows the contact inset. (Actual size.)

Fig. 20 is the wiring diagram, showing the arrangement of the double circuit, and its connection to the different units. It will be seen that the ground wire is common to all tables in the laboratory and that the switch is in the circuit on the "ground side." Referring to the ground wire, and its connection to the chronograph, it will be found connected with the central screw of both units *U′* and *U″* marked *2* and *3* (Fig. 15).

SUMMARY

In the production of The University of Maryland chronograph, the outstanding features of time markers, chronometers, and chronographs, were considered. While their principles were not adopted, the results obtained with them were. The ideal chronograph ought to serve an entire laboratory, in the same way any of the best portable types serve the individual table. This was the goal set, and in a large measure accomplished. Having used the new chronograph in our laboratory of twenty tables, and without an exception, with excellent results, it is only right that it should be used unselfishly for the advancement of laboratory work in other laboratories. With it, it is possible to supply current to any or all of twenty tables, or more if desired, in the form of single induction shocks without interfering with any other circuit. It will supply to one table, one time; to another, a different rate; to still another, both time and stimulation if desired. In other words, no single table in the laboratory is at the mercy of any other. It is capable of doing three independent pieces of work for any one or all tables. It will record time in seconds and minutes, and deliver single induction shocks at a constant rate to the inductorium, at a rate varying from three per second to one in three seconds. This rate can be changed to suit the laboratory requirements, if this rate is not within the range of the needs, with very little trouble, and a few cents expense, or can be designed to fill the requirements without extra expense. It is so constructed, that the units can be fitted with replacement parts, in case a laboratory expands or diminishes. This makes it possible to keep it up to the minute. It can easily be disassembled, only one part being connected rigidly with the propeller shaft. It is positive in execution, adjustable, and foolproof; simple to operate, and durable.

I am indebted to Dr. W. H. Schultz, Professor of Pharmacology, for valuable suggestions.

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THE PHYSIOLOGIC EFFECTS OF THE ELECTROIONIC ADMINISTRATION OF ADRENALIN*

By C. S. Smith, Ph.D., M.D., and J. B. Brown, M.S., Ph.D., Columbus, Ohio

THE introduction of drugs into the body by direct absorption through the skin is usually considered to be a very slow and otherwise ineffective mode of administration. In this regard Cushny[1] may be quoted as follows: "Drugs are also applied to the skin in order to elicit their general action. Volatile bodies are certainly absorbed by it, although much more slowly than by the lungs or by the stomach and intestine. *Solutions in water of nonvolatile drugs are not absorbed from the skin,* but solutions of certain remedies in alcohol, oils, fats, ether, and some other substances which are capable of dissolving or mixing with the fatty covering of the skin, are absorbed fairly rapidly if they are rubbed in thoroughly." Quite recently Kahlenberg, in a very interesting communication,[2] has shown that such substances as urea, cane sugar, salts and borax diffuse readily through dead skin, which, however, is entirely impermeable to them in the living state. Incidentally he pointed out one noteworthy exception in boric acid, which passes through living skin very rapidly, appearing in the urine within a few minutes after the drug is applied to the surface of the body. In most cases, however, the living skin offers an insurmountable barrier to the passage of drugs into the body by simple diffusion.

The use of the direct electric current to bring about the absorption of ionic substances has been known for some time, but, with a few exceptions, the experimental work on this subject has been very indefinite. In 1921 Inchley[3] showed that atropine, strychnine and aconitine of the alkaloids and calcium and ferrous iron of the metallic ions were forced into and through the skin by the application of a few milliamperes of direct current over varying periods of time. Of the negative ions he proved further that cyanide, salicylate and ferricyanide anions were absorbed, but that the penetration was no greater than one would effect by hypodermic injection. Inchley tried adrenalin with no effect on the blood pressure, which is not surprising, since his solution of the drug did not affect the blood pressure of the rabbit which he was using in the experiment when three minims were injected hypodermically. In a very recent article Pack, Underhill, Epstein and Kugelmass,[4] in some excellently controlled experimental work, demonstrated accelerated absorption by electrolysis with the following substances: Hg^{++}, Mg^{++}, Zn^{++}, Pb^{++}, strychnine, pilocarpine, curare, picrotoxin, veratrine, nicotine, cocaine, procaine, butyn, and a number of the halogens, nonmetals and acids. Benzyl carbinol, salicaine, nitrite, salicylate and caffein were not introduced by this method. It seems certain, therefore, that ionic medication with either a positive or negative ion is a possibility, provided practical uses for these ions and this mode of administration are found.

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We have been interested for some time in discovering some simple means for administering adrenalin, particularly for the relief of bronchial asthma. The only satisfactory method is that of hypodermic or intramuscular injection. While the relief with many individuals thus treated is very rapid and complete, repeated application is inconvenient and in many cases impracticable. There is also some danger of overdosage. It should be further noted that very small doses of this drug are effective in relieving asthma, one to three drops of a 1-1000 solution (0.06 to 0.2 mg.) being usually sufficient. It occurred to us, therefore, that, if adrenalin were absorbed at all by ionic medication, this means of introduction into the body would be very desirable, particularly in the treatment of this disease in chronic cases where frequent use is necessary.

Experimental Part

INTRODUCTION

In a preliminary paper[5] an apparatus was described for the electrolysis of an adrenalin solution with deposition of the positive adrenalin ion in the skin. This apparatus was so constructed that, by holding it in the hand and applying the other end of the apparatus to some spot on the body, the circuit was automatically closed. It was simply constructed and could be used conveniently by a chronic asthmatic as often as desired. The area of the positive electrode was about twenty square centimeters. The current was supplied from a small sized radio B-battery, 22½ volts.

In the tests here described a similar apparatus was employed, the only difference being in the size of the positive electrode, which in this case had an area about twice that mentioned above. This was covered with from three to five thicknesses of gauze, which in turn was well moistened with 0.3 per cent adrenalin solution in either hydrochloric or sulphurous acids, sufficient acid being present to keep the solution at a P_H of from 2.0 to 3.0. This low P_H is necessary to inhibit oxidation and coloration of the otherwise unstable adrenalin.

The chemistry of the ionization and electrolysis is as follows: Giving adrenalin chloride the formula, RNH_3Cl, we have the following equilibrium,

$$RNH_3Cl \leftrightarrows RNH^+_3 + Cl^-.$$

In the process of electrolysis the RNH^+_3 gains one electron and is then converted into free adrenalin base and an atom of hydrogen. The depth of penetration of the base is not known. From its effects described below, however, it obviously penetrates as far as the small blood vessels, which it constricts. Whether it diffuses chiefly into the blood vessels and arrives at the circulation by that route, or whether it enters into the lymphatics is not known. Lyon[6] believes that absorption is mainly through the latter channel (with hypodermic injection).

LOCAL EFFECTS

The local effect of adrenalin introduced into the skin by ionic medication is an almost immediate blanching of the area to which the electrode is applied, caused by complete vasoconstriction. The blanching appears in from one to three minutes and persists from fifteen minutes to four hours, depending on

the length of application. The blanched area marks the exact outline of the electrode and does not spread further. In fact, in some cases, in the immediate periphery of the blanched spot, there is a narrow area of hyperemia, suggesting either slight irritation or, more probably, the minimal effect of adrenalin, vasodilatation.

SYSTEMIC EFFECTS

These were determined by taking the systolic blood pressure in the usual manner with a Baumanometer. The subject was caused to lie down during the course of the experiment. A preliminary rest period of at least ten minutes

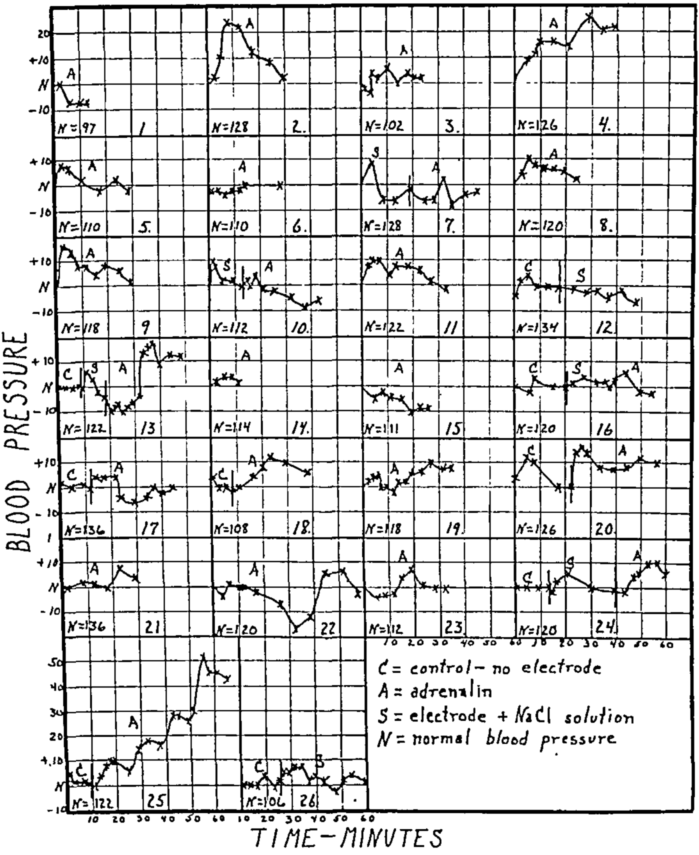

Fig. 1.

was allowed, and then readings were taken over two or three minute intervals until two or more consecutive readings were alike. Then, with as little disturbance as possible, the electrode was applied to the arm, shoulder or chest. Usually several spots of application were made. Controls under like conditions, but with the use of physiologic salt solution at a P_H of 2.0 to 3.0, showed very little if any change from the normal, rarely over five millimeters. The patient was kept as quiet as possible to eliminate the possibility of changes due to excitement. The subjects were, with one exception, men* about twenty years of age. The records of the blood pressure are shown in Fig. 1.

The results of these experiments may be briefly pointed out as follows:

1. Controls(S) in Experiments 7, 10, 12, 13, 16, 24 and 26 in no case showed a deviation of more than ten millimeters from the normal, and in nearly all cases this deviation was on the plus side, i.e., a rise. This indicates that under the conditions of the experiment the electric current alone may produce a slight rise, from 0 to 10 millimeters, and in a few cases a slight fall. In one experiment both conditions existed.

2. During the application of adrenalin, Experiments 2, 4, 8, 9, 11, 13, 18, 19, 20, 24 and 25 gave blood pressure rises of 10 millimeters or greater. This constitutes 46 per cent of the experiments employing adrenalin. The maximum rise was 52 millimeters. This was accompanied by tremors and nervousness, which symptoms persisted for some hours.

3. A number of the experiments showed definite blood pressure lowering with adrenalin, either throughout the experiment, or at some point during the experiment (1, 3, 6, 7, 10, 13, 15, 17, 22, and 23). The maximum lowering was seventeen millimeters, with an average of about five. The lowering was thus not so pronounced as the raising, mentioned previously, although it occurred in almost as many instances.

4. Pronounced raising and lowering occurred in the same experiment in several cases, (3, 13, 17, 22, 23).

DISCUSSION OF RESULTS

The action of adrenalin on the blood pressure is usually stated to depend on the dosage. With minimal amounts of adrenalin the blood pressure is generally lowered. This is explained by Dale and Richards[7] as due to direct action on the lining of the capillaries, resulting in loss of tone and dilatation. With larger amounts of adrenalin, the blood pressure is markedly raised to an extent dependent proportionally upon the amount given, because of profound constriction of the walls of the arterioles. The blood pressure curve of adrenalin administered intravenously shows a sudden, sharp rise, but more gradual fall to normal within a few minutes. The blood pressure lowering curve is similar but reversed. The effect of ionic medication with adrenalin might be expected to show both types of curves, first the lowering of minimal doses, and then a change to a raising as the amount introduced is increased. As a matter of fact the results above show both types, but not generally in the order predicted. Experiments 3, 13, 17, 22, and 23 show a lowering first, and then

*We are indebted to a number of the members of the Sophomore class of the College of Medicine for serving in these experiments.

a rise to a point above normal. The differences between the maxima and minima in these curves varied from 11 to 28 millimeters, which are well out of range of experimental error.* In a number of cases, however, the rise came first, followed by a fall to a point below normal. Furthermore, in some cases only the rise or the fall appeared. From these facts it is difficult, and perhaps not desirable, to attempt to explain these varying actions on pharmacodynamic grounds without additional experimental evidence.

The differences are undoubtedly due to variations in individual reaction towards adrenalin and in the rate of absorption of the drug. This latter would in turn vary directly with any of the conditions affecting the electrical conductivity of the skin or even of the body in general through which the current must pass.

These experiments show in two ways that appreciable doses of adrenalin may be introduced into the body through the skin by means of the electric current. In the first place, in every experiment the local blanching of the skin occurs. The electrode alone with salt does not produce this effect, nor is there any sign of blanching when adrenalin, dissolved in water or in an unctuous medium, is applied to the skin. Likewise with reversal of the polarity of the electrodes the results are negative. All of these facts prove that the electric current is essential in overcoming the natural barrier which living skin opposes to the diffusion of adrenalin chloride. In the second place, in the majority of cases, enough adrenalin finds its way into the system to produce appreciable changes in the blood pressure, either lowering or raising it. While in a few instances this might be ascribed to the effect of the current alone or to individual excitability, in many cases the effect is much greater than we have been able to produce by the current alone. In these cases the effect is due to a general systemic action of the adrenalin.

SUMMARY

The effects of the electronic administration of adrenalin on the human body may be stated as follows:

1. Blanching of the skin occurs at the point of application of the electrode.

2. The blood pressure effects are variable. In almost half of the cases a rise of ten millimeters or more is observed. A rise of as high as fifty millimeters may result. In a few cases there is a distinct blood pressure lowering, thus showing the effect of minimal doses.

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*The limit of error in our readings was not more than ± 1 to 2 mm.

AN APPARATUS FOR THE ELECTROIONIC ADMINISTRATION OF ADRENALIN*

By J. B. Brown, M.S., Ph.D., and C. P. Effler, Columbus, Ohio

An article by Pack, Underhill, Epstein and Kugelmass† on electroionic medication suggested to us the possibility of applying this principle to the administration of adrenalin. Such a mode of administration would overcome the disadvantages and inconveniences of hypodermic injection and would be especially desirable where frequent application is necessary for either local or systemic effects.

With these points in mind we have devised an instrument, a cross section of which is shown in Fig. 1. The metal parts are of nickeled brass. Electrode *A* (–) is of the size and shape of an ordinary door knob. The second electrode, *C* (+), consists of a flat disc, grooved on its outer edge so as to afford a concave

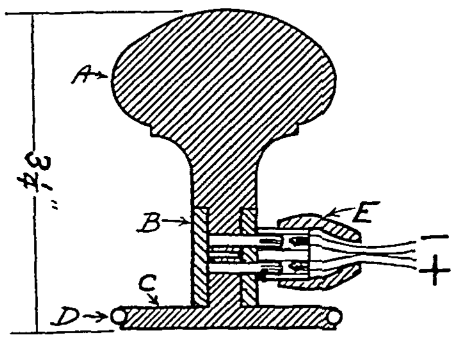

Fig. 1.

pocket for a broken ring of spring brass, *D*, which holds in place several thicknesses of gauze over the lower surface of *C*. The exterior of *D* is lacquered with an insulating material. *B* is a rod of fiber, bakelite or hard rubber which separates and insulates the electrodes. *E* is a small electric plug leading by a cord to a small radio *B*-battery (22.5 volts), and is provided with a third pin, not shown, between *B* and *E*, making it impossible to interchange the polarity of the electrodes after the proper battery connections have been made.

The instrument is used as follows: 4 to 6 circular pieces of gauze of a diameter about one-half inch larger than the disc *C* are placed on *C*, and the spring *D* is pushed on to hold them in place. The apparatus, ready for use, is pictured in Fig. 2. The gauze is well moistened with adrenalin solution (either the chloride or sulphite). The electrode *A* is then firmly held by the patient in one hand and applied to the part desired. In the treatment of asthma

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and for blood pressure effects we have found the arm and shoulder to be satisfactory points of application. In this way the patient completes the electric circuit through his own body, and the positive adrenalin ion is deposited on and absorbed by the negative surface to which *C* is applied. The local effect is chiefly a complete blanching of the skin. The discomfort is practically negligible and consists of a slight tingling in the hand holding the apparatus.

Preliminary experiments have shown that solutions of adrenalin from 0.1 to 1.0 per cent in strength produce the local constriction. The optimum concentration is about 0.3 per cent. The P_H of the solution should be low, preferably from 2.0 to 3.0, in order to increase the stability of the adrenalin and inhibit its discoloration. The instrument is used until the desired effect is

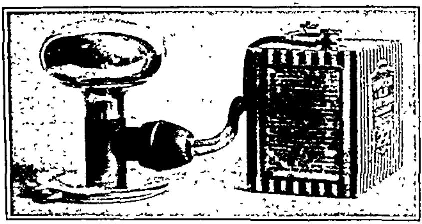

FIG. 2.

obtained, usually from five minutes to an hour. The gauze need not be changed for several days, when the color becomes unsightly. When not in use, the gauze may become completely dry without impairing the adrenalin, in which case it is moistened again with water. One charge, seven cubic centimeters of 0.3 per cent adrenalin, contains about twenty normal intramuscular doses. The charge is shown to be exhausted when the local effect is no longer produced.

A series of experiments upon the blood pressure effects of this method of administration of adrenalin in normal individuals is now in progress. In addition, this method is being tried clinically in selected cases of bronchial asthma. The results of these observations will be reported in the near future.

LABORATORY METHODS

A METHOD FOR THE STUDY OF THE VARIATIONS IN VOLUME OF ORGANS IN THE INTACT ANIMAL*

By Estes H. Hargis, M.D., Rochester, Minnesota

No comprehensive study of the variations in volume of organs in the unanesthetized animal, other than the limbs, has been made. Many plethysmographic studies are recorded for which the animal was anesthetized, but under such circumstances repeated observations for any length of time are not possible, and no results uncomplicated by the effects of the anesthetic can be obtained.

In a problem on the variation in volume of the spleen in relation to digestion and other processes, in which it was desired to make repeated observations extending over long periods, a procedure which would not be complicated by an anesthetic was necessary; therefore, the method here described was formulated. While the studies on the spleen were of paramount interest, the procedure was also applied to the kidney and gall bladder as a control. A plethysmograph for this purpose must be light, and flexible enough to permit of its being sutured into position and rigid after suturing. It must be of material that can be sterlized, and it must be nonirritating so that it will heal in position. Such an instrument was made of nonflexible collodion. Plethysmographs for the various organs demand special attention only in the construction of the model.

CONSTRUCTION OF THE SPLENIC PLETHYSMOGRAPH

Anatomically the spleen of the dog lends itself to a study of this kind. It is mobile; its vessels and omentum permit it to be drawn to the surface through an abdominal incision so that subsequent procedures are comparatively simple. Its long axis runs obliquely downward and forward, and the greater end or head is nearer the median line and slopes toward the tail, which, however, is somewhat larger than the median portion of the organ. The width is greater than the thickness, and the vessels are given off in longitudinal group along the dorsal surface.

The first step in the construction of a plethysmograph, which can be placed around an organ in the abdominal cavity and heal in position, is to mould from warm paraffin a model of an organ similar to the one to be studied (Fig. 1). Such a model can be made from the spleen of an animal of approximately the same size. As the size of the spleen varies consider-

*Work done in the Division of Experimental Surgery and Pathology, The Mayo Foundation.
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ably in dogs of the same weight, it is necessary to have two or three plethysmographs of different sizes before attempting to apply one. The model should be approximately one-fourth larger than the organ, which will thus fill about 80 per cent of the completed instrument. Allowance should be made for the contraction of the collodion during the process of hardening. Paraffin is placed in warm water until it becomes soft; then the model is moulded into the desired shape with the fingers (Fig. 2). It conforms to the shape of the spleen in a general way, and is shaped something like a flattened banana. The concave surface represents the hilar or dorsal surface

Fig. 1.—Paraffin model to be used in the preparation of a collodion plethysmograph for the spleen of a dog.

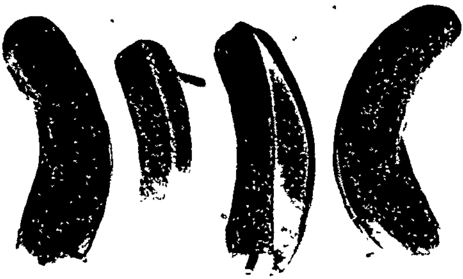

Fig. 2.—Splenic plethysmographs of various sizes. One has been opened and sutured, and another is opened along the convex surface to show the interior of the instrument.

of the spleen; the convex, the ventral. The two other faces, flattened longitudinally, will be referred to as the superior and inferior surfaces. Two ends represent the head and tail. When the model of the organ is thus complete, a shelf or ledge, triangular in cross section and with an altitude of 1 cm., equidistant from the superior and inferior surfaces, beginning about 2 cm. from the ends on the convex surface, and running around both ends and the entire length of the concave surface, is moulded from a separate piece of paraffin. This shelf can be trimmed to the desired shape with a knife. A piece of glass tubing, of not less than 6 mm. inside diameter, with relatively thin walls, is introduced into the paraffin deep enough to make it rigid and

allow at least 4 cm. to protrude from the surface. This tube should be placed obliquely to the surface about 5 cm. from the lesser end.

After the model is completed it is placed in cold water and allowed to harden, is then removed and thoroughly dried. Several thick layers of non-flexible collodion are applied to the surface, either by pouring from a suitable container and distributing with the fingers, or by dipping. This should be allowed to remain unmolested until the collodion has become dry and hard. Then three layers of gauze are fitted snugly about the model and successive layers of collodion added to a thickness of approximately 2 mm. The glass tube need not be covered with gauze but added layers of collodion are necessary to make its mould sufficiently rigid after the glass has been broken out.

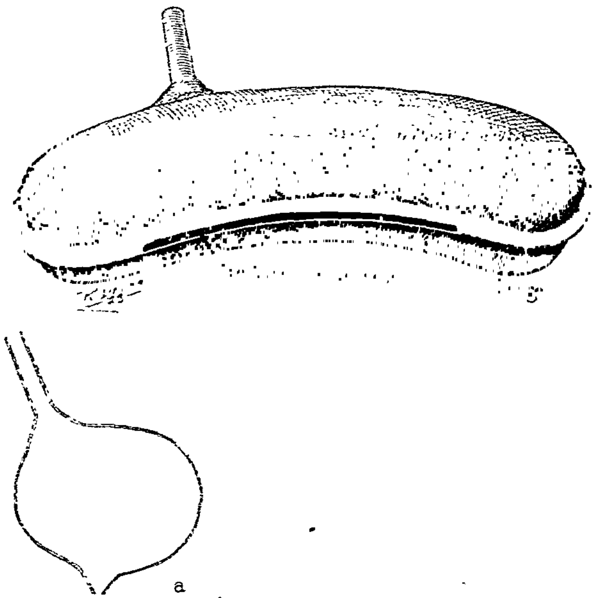

Fig. 3.—Completed plethysmograph showing ledge and aperture for vessels; *a*, cross section of instrument at point of exit of tube.

At least forty-eight hours should be allowed for the collodion to harden. After it has become thoroughly dry and hard an incision about 2.5 cm. in length is made longitudinally along the convex surface, and the model is placed in a bath of boiling water until the paraffin has melted out. The plethysmograph is taken from the water-bath while hot and rubbed vigorously with gauze to remove any paraffin adhering to its surface, and, if necessary, is immersed in a second bath of boiling water. After it has cooled for a time, the glass tube is broken out by placing it on a metallic surface and tapping gently with any small instrument. The plethysmograph should be opened with a sharp knife along the apex the entire length of the ledge, and the edges on either side of the opening cut away with scissors, beginning about 2 cm. from either end on the concave side, to provide an aperture of sufficient width to accommodate the vessels (Fig. 3). A distance of 2 or 3 mm. on each side of the slit is sufficient for this. The sharp edges are covered with collodion and allowed to dry. A thick-walled rubber tube, about 10

cm. in length, is fitted to the collodion tube of the instrument, and if there is any doubt about its remaining in place it may be sutured with a small needle and silk. The small opening on the convex side of the instrument should be sutured and covered with a layer of gauze and several layers of collodion. The plethysmograph is now ready for sterilization and may be boiled repeatedly without injury.

METHOD OF APPLYING PLETHYSMOGRAPH TO THE SPLEEN

All operative procedures are performed under complete anesthesia and rigid sterile technic. Plethysmographs of at least three sizes are sterilized by boiling, and two sterile test tubes are filled with collodion to be used during the closure of the instrument. The abdomen is opened through a left rectus incision and skin towels are applied. Although obvious, it may not be amiss to emphasize the need for the utmost care in introducing into the abdominal cavity a foreign body which is to be left for a long time if it is not to cause symptoms or discomfort. If moderate care is exercised good results will be obtained. The spleen is exposed, drawn out of the wound, and surrounded with warm saline packs, and a layer of omentum is fitted around it. A plethysmograph of appropriate size is selected and the omentum-covered spleen is introduced. The splenic omentum and vessels are arranged so that they are well inside of the space provided for them. The spleen, in the contracted state which exists during operation, should fill about eighty per cent of the instrument and the cut edges of the plethysmograph should fit snugly around the splenic omentum, without, however, any encroachment on the lumen of the vessels. If the size of the plethysmograph is satisfactory and the vessels are properly cared for, the instrument may be closed; this is most easily accomplished by the use of silk and a small needle with a cutting edge.

The method of suturing is optional, but whatever method is chosen, the first suture at each end should be placed at the edge of the splenic omentum in a manner to produce a snugly-fitting angle. The sutures are carried toward and around the ends as far as the instrument has been opened. It is rarely necessary to place sutures through the portion receiving the omentum and vessels unless there is an ill-fitting area, in which case one or more interrupted sutures may be used to approximate the edges, care being taken not to injure the vessels. When the suturing has been completed the instrument is thoroughly packed off with large gauze sponges, and several layers of collodion are applied to the suture line and allowed to dry for several minutes. A thin strip of gauze is placed over this surface and in turn covered with collodion and allowed to dry.

The omentum is drawn around the plethysmograph in such a manner as to enclose it completely, leaving no gaping edges or openings, and is gently sutured in place with 00 catgut. The instrument is placed in the abdomnal cavity and the rubber tube brought to the surface through a stab wound high on the left side; in this position it is more difficult for the animal to remove it, but at the same time the position must not cause torsion of the vessels and interfere with the circulation of the spleen. Tension is exerted

on the tube from the outside to draw the plethysmograph firmly against the abdominal wall so that adhesions may form. Unless this precaution is observed infection may later creep in around the tube. At least one layer of nonabsorbable suture material is used in closing the wound. The rubber tube is cut about 5 cm. from the margin of the skin and left open.

Precaution must be taken to prevent the animal from removing the tube. A wide, closely-fitting metal collar has been found quite satisfactory, and causes the animal no discomfort. A week should be allowed before plethysmographic observations are made. By this time, if care has been taken, there is no danger of leakage from the plethysmograph into the abdomen.

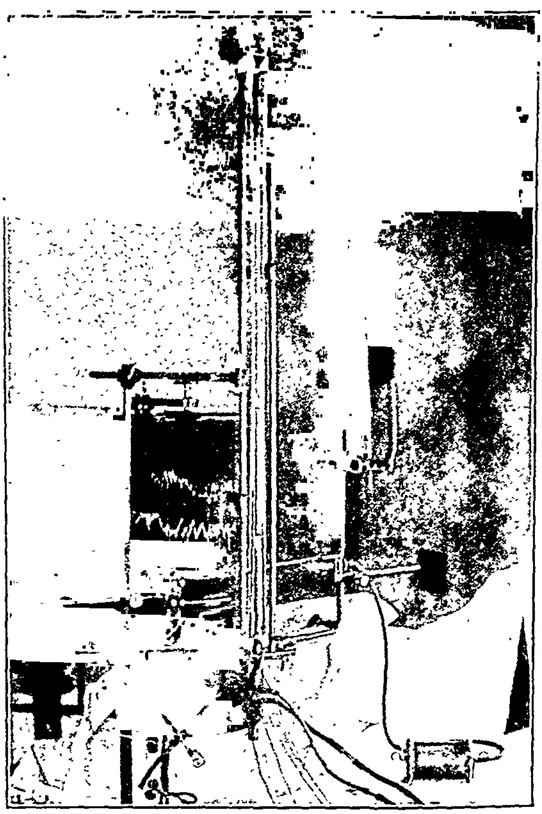

Fig. 4.—Water manometer and method of recording.

METHOD OF RECORDING OBSERVATIONS

Observations are made only on animals that have been thoroughly trained for the purpose. They receive no anesthetic or drugs, and suffer no discomfort. At the end of the first postoperative week they are placed under preliminary observation for increasing periods of time so they may become accustomed to the surroundings and acquire the habit of lying quietly on the table. They are easily trained, and learn the routine quite readily; the trained animal will remain perfectly contented for long periods, frequently sleeping while the observations are being made. The animal is placed on his side upon a concave holder lined with soft mats, a collar attached around

the neck, and the feet restrained lightly. Appropriate connections are made between the tube from the plethysmograph and the recording apparatus.

The water manometer, with a special method of recording, has been chosen as the best method for recording changes in volume of the spleen. The tambour has been used with very good results, but its use has some disadvantages which are not encountered in the former method. It is essential to have a method in which there is as little resistance as possible in the recording apparatus, so that it will react quickly to changes and will be capable of recording the delicate variations in the volume of the organ. A method of recording to be used with the water manometer[1] (Fig. 4) consists essentially of sections of glass tubing, with an inside diameter of 3 mm., placed in a brass frame so that each section is in front of a vertical column. On each of the three similar columns there is a delicate carriage which may be moved up and down, having on the drum side a writing point and on the

Fig. 5.—The effect of rebreathing on the volume of the spleen in the intact animal is obtained by the method described. The upper curve is splenic volume; the median curve, respiration recorded by the respiratory tambour, and the lower curve, the time in seconds.

observer's side a pointer which is to be kept on a level with the meniscus of the column of the fluid by manipulation of the carriage with two cords. The recording instrument is held in position by clamps and so placed that the writing point rests on the revolving surface of the smoked drum. This method of recording has proved very satisfactory and one is able to obtain consistently records which are clear and accurate (Fig. 5).

DISCUSSION OF METHODS

By the methods formerly used studies on the changes in volume of the internal organs were difficult or impossible because results could not be obtained without being complicated by the effects of an anesthetic; the results of external stimuli could not be investigated, because each experiment was an acute experiment and the animal could not be studied for any extended

[1]Potter, J. C.: A Mechanical Method for Recording Small Variations in Pressure and Volume, Jour. Lab. and Clin. Med., 1924, ix, 648-650.

Our experience with this test agrees with that of Bernheim, particularly in hepatic conditions where it serves to indicate the extent of impairment in biliary function and also the response to treatment. Thus, in jaundice with a high index, decrease in the index denoted improvement before changes in the color of the skin were detectable. Patients with cholecystitis had indices varying from normal up to fifteen or more, the amount of increase above normal indicating the extent of impairment of the biliary function. The index was increased in hepatic cirrhosis, being marked in the biliary types and only moderate in the portal and cardiac types. Malignancy of the liver always produced a high index, the extent being dependent upon the amount of hepatic involvement and the presence or absence of jaundice; thus the highest index in our series was present in a patient with advanced hepatic carcinoma with intense jaundice and an index of 150.

It is evident from the above results that the icterus index may serve both as a useful clinical index of the extent of impairment of biliary function and also as a quantitative measure of the progress of restoration of this function, particularly in jaundice. In the latter condition we have been considerably aided by the van den Bergh test used in a qualitative way to differentiate functional or hemolytic from obstructive jaundice. In this manner, by a combination of these two tests, one quantitative and the other qualitative, we have obtained considerable information concerning the biliary function of the liver.

DYE TESTS

The phenoltetrachlorphthalein test of Rosenthal[10] needs little description or comment, much having already been written concerning it. We have followed the technic originally described by Rosenthal with only slight modifications: 5 mg. of the dye per kilogram body weight were injected and the percentage present in the serum at the end of fifteen minutes, one hour and two hours (if indicated) was determined, using the colorimeter devised by Rosenthal. In cloudy sera or in sera with traces of hemolysis, we have used acetone to extract the color, as suggested by Bloom and Rosenau.[11]

Early in our experience we found that the injection of 40 to 50 c.c. of a solution containing 5 to 8 c.c. of dye, particularly in patients with marked jaundice, cachexia or cardiac disease, caused at times severe reactions, but this has been obviated by the injection of the concentrated dye. Our results with this method have been uniformly good. The percentage of dye retention was proportionate to the degree of liver dysfunction, reaching up to 35 per cent in the severe types. Thus the test seems to be a useful clinical index of hepatic function. It is not, however, without its drawbacks: first, the injection of so much dye into a patient whose liver is already damaged is not always safe. Furthermore, since it never reaches concentrations in the blood stream over 35 per cent even in the severest cases, its greatest value is in the severest grades of hepatic involvement. Finally, as pointed out recently by Maurer and Gatewood,[12] the dye is not wholly eliminated by the liver.

Appreciating these facts, Rosenthal studied other dyes and recently proposed bromsulphalein,[13] which is nontoxic, is more rapidly eliminated by

the liver, and, if retained in the blood as a result of liver impairment, is in much higher concentration, the percentage being used as a numerical index of the extent of liver dysfunction. The technic of the test is so simple and safe as to lend itself readily to clinical use; only 2 to 3 c.c. of the dye need be injected and it is necessary only to draw the blood at the end of thirty minutes to obtain the result. Normally no dye should be present in the blood at thirty minutes; the percentage of dye retained at this time represents the extent of impairment of liver function.

Our experience with this newer method of Rosenthal is too brief to warrant conclusions but even this has been sufficient to prove its great superiority over the tetrachlor method and to indicate that it is a valuable test well adapted to clinical use. Whether or not it will serve to indicate minor variations in hepatic function remains for further investigations to disclose.

We must not forget that any dye test simply measures the ability of the liver to extract this foreign substance from the blood stream and get rid of it in the bile; it is in reality a test of liver permeability and not of its total function. For example, even though the dye test may show 80 per cent impairment, it is possible that the carbohydrate or nitrogenous functions may be only slightly affected; in this instance the 80 per cent may give a false impression. We cannot therefore agree with the statement of Rosenthal that the percentage of dye retention measures the extent of liver impairment; it simply represents the degree of impermeability which may in turn be taken as a rough index of impairment of total function, since it is highly probable that any damage to the liver cells will result in impairment of the permeability.

NITROGEN PARTITION STUDIES

A low percentage of urea nitrogen with comparatively high percentages of ammonia and amino-acid nitrogen in the urine as compared with normal urinary nitrogen partition figures, was considered by earlier investigators as evidence of organic liver damage. Similar attempts were later made to study in a comparative way the urea, ammonia, amino-acid, and nonprotein nitrogen content of the blood. Normally, urea nitrogen makes up from 40 to 60 per cent of the total nonprotein nitrogen of the blood; when it falls below 40 per cent, and particularly if there is coincident increase in amino-acid and ammonia nitrogen, it is indicative of liver damage.

The value of blood nitrogen partition studies in patients with liver disease has hitherto received scant attention, judging from the paucity of literature on this subject. Our experience with this method has been confined chiefly to the estimation of urea nitrogen as compared to the total nonprotein nitrogen in patients with various liver conditions, and no attempt has been made to study amino-acid and ammonia nitrogen figures in these patients. In patients with advanced liver disease, low urea and comparatively high nonprotein nitrogen values were obtained, but, in patients with lesser grades of hepatic disease, the proportion was usually within normal limits. It is evident from this that, as with the levulose tolerance test, studies of blood nitrogen partition will be of little value from a practical standpoint

clinically, and, even where gross organic damage exists, the technical details necessary for its performance hardly warrant its clinical use. It is possible that similar factors of readjustment as discussed under levulose are at work here and only when the damage is too great to be overcome by other factors does it show itself in the partition figures. So little is known of the mechanism of protein metabolism that we believe it of value to continue such studies in patients with various hepatic conditions, and, even though it may be of little value clinically, it may help to solve some of the complex problems of liver physiology.

COMPARATIVE STUDIES

In addition to trying out separately each of the above-mentioned functional tests, we have attempted to compare these tests by carrying out as many of them on the same patient as was possible, being careful to do them closely together in order to preclude any variability in liver function. Our results bear out in a general way the conclusions drawn above concerning each individual test. Probably the greatest similarity in quantitative results existed between the icterus index and the dye tests, particularly in mild dysfunction. This is readily explained when one considers that both of these tests are really dependent on liver permeability. The levulose tolerance and dye tests corresponded fairly well but only in those patients in which marked organic damage to the liver existed. In the milder cases the levulose tolerance often gave a normal curve or showed only a slight delay in return to normal.

We do not believe that this is the fault of the test. It is highly probable that in these patients there exists little damage to the carbohydrate mechanism or possibly its damage has been adjusted by other factors. So little is known as yet of the actual mechanism of carbohydrate metabolism that this must, of necessity, be speculative. The facts given concerning the levulose tolerance apply in a general way to tests for disordered protein metabolism in the liver. Only in gross organic liver disease does one find differences between nonprotein nitrogen and urea nitrogen such as has been described as probably indicative of protein dysfunction. In minor degrees of dysfunction these methods do not detect any deviations. This is also true to a certain extent with the hemoclastic, but here we have an additional factor in that sometimes positive reactions are obtained in patients who show no other evidence either clinical or by other functional methods, of hepatic dysfunction.

CLINICAL FORMULA

As a result of these studies with the various tests, it seemed to us that in order to obtain a complete idea of the hepatic function present in any patient, it is necessary to carry out on that patient all these tests, since each concerned itself with a different function. The objections to such a procedure are at once obvious, the annoyance to the patient, the possible difference in function if the tests are done on different days, etc. It occurred to us therefore that if these tests could be combined in such a manner as to be done at one time

without losing their sensitivity it might yield valuable information and make for a complete study. The following procedure was accordingly adopted:

The patient is prepared as for a levulose tolerance; on the same tray the calculated amount of dye contained in a syringe is also prepared. Blood is then withdrawn into two tubes, one citrated or oxalated, and one a plain tube. Enough blood is withdrawn to suffice for all these tests. Through the same needle the calculated amount of dye is injected. Immediately following this the patient is instructed to drink the levulose solution. Blood is then withdrawn from a vein of the opposite arm at thirty minutes, one hour, two hours and, if necessary, three hours after the injection, the blood being again drawn into plain and oxalated tubes as indicated. The blood is allowed to clot, is centrifuged, and the serum is pipetted off. The serum collected before the injection is then used for the icterus index determination and also as the standard for the dye test. As a rule 2 to 3 c.c. of serum is sufficient for this purpose. The oxalated blood collected before injection is used for the urea nitrogen, nonprotein nitrogen, and sugar determination. Blood sugars are then done on all the bloods (oxalated) taken subsequent to injection, and the sera are used to determine the dye retention as described in the Rosenthal method.

By this procedure we are able to carry out the icterus index, blood chemistry, levulose tolerance and dye tests, at one time and thus gain information concerning the bile-pigment, excreting functions of the liver and also its carbohydrate and protein metabolic functions.

Our series of combined tests, though small, has been sufficient to prove that by this method complete insight into the total known hepatic functions may be obtained and properly evaluated. Although at present from a diagnostic standpoint the dye tests and icterus index are most valuable in estimating to some degree liver dysfunction, yet by studying hepatic conditions by the simultaneous combined method, we hope to collect enough data for later report, tending to arrive at a better understanding of normal and abnormal hepatic physiology.

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DISCUSSION

Dr. Ralp G. Stillman.—I think Dr. Rubenstone emphasized the difficulty of getting any satisfaction in such an active organ as the liver. In all of these functional tests we have to have a very marked reduction in function before any of the tests we have will indicate it. None of the tests that we have made have been wholly normal in carcinoma involvement of that organ.

A SIMPLE COMBINATION DISTILLATION AND AERATION APPARATUS FOR THE MICROESTIMATION OF NITROGEN*

By Robert F. McCrackan, A.M., Emanuel Passamaneck, B.S., and Kate E. Harman, B.A., Richmond, Va.

Many papers have been published recently containing suggestions for overcoming difficulties inherent in the original Folin and Wu[1] micromethods for determining nitrogen. The troubles most frequently mentioned are: bumping and formation of silica during digestions; bumping, frothing, and drawing back of the distillate during distillations; and cloudiness and variation in shades of colors after nesslerization. Folin,[2] himself, has suggested centrifuging, to get rid of turbidity, preparatory to direct nesslerizations, and he advises that test tubes be thoroughly dried to reduce the danger of bumping during digestions.[3]

Watson and White[4] devised a special delivery tube to prevent the froth from urease solutions passing into the distillates. Youngburg[5] advocates the use of "open delivery tubes" in microdistillations as a means of preventing distillates being drawn back into distillation tubes. Moore and Jones[6] recommend passing air from an inflated rubber bag through the urease solutions during distillation, and through digestion mixtures until half evaporated. Boggs and McEllroy[7] pass a slow current of air through the descending part of the delivery tube during distillations. Weathers and Sweany[8] have described apparatus for running eight aerations at once. Wishart[9] proposes the use of beads in a constricted urease tube as a remedy for frothing, and he admits sodium carbonate into the distillation tube through a special tube after the distillation is well advanced. Horvath[10] uses a safety tube with a pinchcock that can be opened when the distillate is being drawn back. Hindmarsh and Priestley[11] claim they had no trouble from frothing when they caused a current of dry air to impinge on the surface of the borax solution to drive the ammonia over in a urea determination on 0.1 c.c. of blood. Osborn[12] describes apparatus for completing four digestions at once, after having carried each separately to the point where the mixture turns brown. Johnson[13] proposes the use of a "flutter valve" to prevent the drawing back of distillates. Parnas and Heller[14] propose the distillation of the ammoniacal solutions under reduced pressure with steam, and that condensations be made with a silver cooler. Stanford[15] uses a closed vacuum system and brings about distillation at about 35° C. Koch and McMeekin[16] propose modified reagents for digestions and for direct nesslerizations. Feinblatt[17] thinks satisfactory results can be obtained by the nesslerization of urease solutions without distilling.

The apparatus described here has been tried out by about a hundred students, teachers, and clinicians, over a period of about two years. It has been

*Contribution from the Medical College of Virginia, Laboratory of Biochemistry.
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found satisfactory because of its simplicity, and because it eliminates so many troubles, and makes it possible for people of medium ability to get trustworthy results without a great deal of practice. The apparatus is designed to obtain the ammonia from an ammonium salt by distillation, or aeration, and with a view to estimating it quantitatively in the distillate.

An Erlenmeyer flask, *A*, Fig. 2, clamped in position on wire gauze, and filled a third or less full of distilled water containing one or two drops of concentrated sulfuric acid, is used as a steam generator. It has a safety tube, *B*, at least thirty inches long, which is slightly constricted at its lower end. A test tube is sometimes hung over the upper end to prevent the entrance of dust. A delivery tube, *D*, constricted a little at its lower end, leads through the rubber stoppers, *C* and *E*, from flask, *A*, into a tube or flask attached to rubber stopper, *E*. Similarly a second delivery tube, *F*, with a much constricted outlet, leads through rubber stoppers, *E* and *G*, into dilute acid in a tube or volumetric flask, *I*, which is attached to rubber stopper, *G*. The tube, *H*, that leads from *I*, may

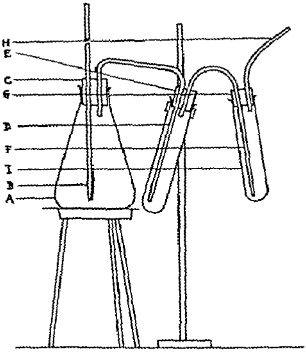

Fig. 1.

be attached to a pump, if the apparatus is to be used for aerating. It can be further improved as aeration apparatus by making the delivery tube, *F*, from one of Folin's thirty cm. aeration tubes, which ends in a bulb with numerous perforations, and it can be still further improved by causing the air entering flask *A*, to pass through another of these tubes substituted for tube *B*. This can be accomplished by removing *B*, or by capping *B* with a piece of rubber tubing plugged with a glass rod and having the Folin tube pass through a third hole in stopper, *C*, into the dilute acid in flask *A*. In such an arrangement it would only be necessary to remove the rubber cap from one of the tubes leading into flask *A*, to the other, to change an aeration outfit into one for distillation, or vice versa.

It is the practice in this laboratory to follow Folin's methods with very little variation, except that the ammonia formed in total nitrogen determinations is distilled before nesslerization. Five c.c. of ten normal sodium hydroxide (40 gm. per 100 c.c.) is used to liberate the ammonia in a total nitrogen deter-

mination, and saturated borax, as directed by Folin, in urea determinations. The usual 75 c.c. test tube has been found satisfactory from which to distil the ammonia from a digested mixture, but a flask is better where the ammonia has to be distilled from a urease solution. This is because of the excessive frothing. When a test tube is used, Fig. 1, the delivery tube, *D*, may be bent in such a way as to cause the bottom of the test tube to rest on the edge of the gauze that supports the flask *A*, making it possible to heat both with one burner. When a flask is used on stopper *E*, results are more liable to be high, because of the greater amount of ammonia in the air. If the flask is large, or if ammonia fumes in the laboratory air are appreciable, it may be found desirable in accurate work to acidify the ammonium salt solution, if it is not already acid, so as to insure retention of the ammonia, and then to displace the air in the flask with steam from generator, *A*, then to block the tube, *F*, with a tube of distilled water, while the flask cools and fills up with ammonia-free air from flask *A*.

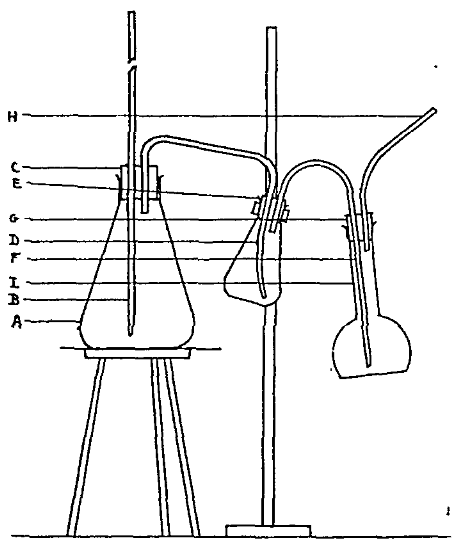

Fig. 2.

The receiver containing an acid solution can be attached at *G*, and the ammonia be liberated and distilled in the usual way.

The advantages to be obtained in the use of this apparatus are:

1. It eliminates bumping and back-suction during distillation.

2. It reduces the frothing evil of the urease method, as a larger container than a 75 c.c. test tube can be used, and the burner may be removed at any time without spoiling a determination.

3. Constrictions in tubes, *B* and *F*, or bulbs at their ends, with perforations, insure better absorption of ammonia from air bubbles.

4. Distillation removes most of the causes of cloudiness in nesslerized solutions. The troublesome silica, dissolved from the container during digestion, the salts resulting from the nesslerization of concentrated acids, the catalyzer, and magnesium compounds that Folin thinks largely responsible for clouding during nesslerization,[18] are left behind, so that the distillate unknown can be

nesslerized exactly the same way as the standard known. It is unnecessary to add anything to the known before nesslerizing to insure the same shades of color and parallel conditions in the known and unknown, and only a third as much Nessler's solution is used as when digestion mixtures are nesslerized directly. Indeed, Folin's own ideal conditions for using 10 c.c. of Nessler's solution per hundred c.c. of nesslerized mixture, in the absence of interfering acids and alkalies, are obtained.

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ACETONE METHYLENE BLUE—METHYLENE AZUR EOSIN SOLUTION FOR STAINING BLOOD SMEARS*

By Frederic Proescher, M.D., and Albert S. Arkush, A.B., Agnew, Calif.

METHYL alcoholic solutions of eosinic acid methylene blue (Jenner, May-Grünwald) in combination with methylene azur (Leishman, Wright) are the commonly employed routine blood stains. The commercially prepared stains may vary greatly in their staining properties because of the difficulty in preparing an eosinic acid methylene blue methylene azur of constant proportions. Different batches vary greatly in their staining effects. McNeal has lately advocated a methyl alcoholic solution of eosin, methylene azur, and methylene violet (tetra chrome) as a substitute for Leishman's stain. While this stain is fairly good, it cannot compare with a good Leishman or Wright stain.

We have tried various combinations of eosin, methylene blue, and methylene azur in methyl alcohol and acetone. By using acetone (and water) as a solvent, we find that the granules are excellently stained and precipitation of the dye is reduced to a minimum.

The stain† is prepared by mixing equal parts (about 60 mgms. each) of methylene blue (Merk), thiazin red (methylene azur**), and eosin with 25 c.c. water, and then adding 50 c.c. acetone. The acetone should be C.P., Mallinckrodt's being used by the authors. Toluidin blue may be used as a substitute for methylene blue. Gruebler's eosin (extra BA) should be used if obtainable. The domestic eosin Y. of the National Aniline and Chemical Company is not so good, though usable. By substituting orange G. for part (about ⅓) of the eosin, the eosinophilic granules are made slightly more prominent.††

The ranges of time given above cover optimum periods for various makes of dyes and slight changes in concentration. The proper intervals for the stain on hand should be determined by trial each time it is made up, and care taken thereafter that the acetone does not evaporate. It may also be noted that in certain cases of pathologic blood, a longer period sometimes gives a much better result.

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*From the Pathological Laboratory, Agnew State Hospital, Agnew, Calif.
Received for publication, Sept. 25, 1925.

**The methylene azur was prepared by oxidation of methylene blue with sodium peroxide.

†Cover the smear with the stain, and allow the adhering solution to remain at least 30 seconds. Wash with tap water. The staining effect is due to evaporation of the acetone. If the stain is not shaken from the slide, it must be allowed to stand several minutes and then diluted as with the Wright method. Care should be taken that the acetone does not evaporate from the staining solution.

††As a substitute for the above offered staining solution we have lately used the "Soloid" tablets. The Romanowsky stain (Wright's modification) gave the most satisfactory results. Two tablets are ground in a mortar and 10 c.c. of 75 per cent acetone added, the solution is filtered and ready for use. The smear is covered with a few drops of the stain, the excess stain is shaken off, and the smear allowed to stand from 2 to 3 minutes, then washed with tap water.

The resulting picture compares very favorably with Wright's or any of the other conventional blood stains, and is very similar to them in its effect. The granules, and particularly the neutrophilic granules, are brought out very distinctly. This is especially noticed in some pathologic blood smears, as in leucemia. The red cells show a pale green color* in thin smears, dark green in heavy smears.

Additional advantages of this stain are its rapidity, ease of preparation, its economy, and the fact that no buffer is necessary for dilution or washing.

A SIMPLIFIED MEDIUM FOR THE CULTIVATION OF ENDAMEBA DYSENTERIAE†

By Charles A. Kofoid, Ph.D., Sc.D., and Edna Hannibal Wagener, A.B., A.M.
Berkeley, Calif.

BOECK and Drbohlav[1] have recently described a method for the cultivation of *Endameba dysenteriae* on a medium composed of a coagulated egg slant covered with 10 c.c. of Locke albumen, prepared by shaking the whites of two eggs in 1000 c.c. of Locke's solution and passing the whole through a Berkefeld filter in order to sterilize it. Another medium is made by covering the egg slant with 1 part of human serum and 8 parts of Locke's solution.

In carrying on a series of experiments on the growth of *Endameba dysenteriae* in different kinds of media we have found that either rabbit, rat, cat, or guinea pig blood may be substituted for human serum. The most satisfactory medium was found to be the coagulated egg slant covered with 10 c.c. Locke's solution, containing 0.5 per cent of defibrinated rabbit blood.

This medium is less favorable to the bacteria, which are found with the ameba cultures and quickly lower the P_H, than either Boeck's egg-Locke-serum medium or his egg-Locke-albumen medium. It is also simple to prepare, as fresh, sterile rabbit blood can be easily obtained.

On our rabbit blood medium the endameba multiply rapidly and are frequently found ingesting rabbit red blood cells. It is necessary to transplant the cultures every forty-eight hours in order to keep them going continuously.

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*Sometimes light brown, depending upon the concentration.

†From the Zoological Laboratory, University of California, Berkeley, California.

Received for publication, Oct. 24, 1925.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

Stander, H. J., Duncan, E. E., and Sisson, W. E: Chemical Studies of the Toxemias of Pregnancy. Bull. Johns Hopkins Hosp., June, 1925, xxxvi, No. 6, p. 411.

The elaboration of a completely satisfactory method for differentiating between the various types of late toxemias of pregnancy and the discovery of the etiology of eclampsia are among the leading unsolved problems of the field of obstetrics.

The authors review the blood chemistry studies which have been made in these fields and report their own studies of the blood and urine of normal nonpregnant, normal pregnant, and abnormal pregnant women.

The pregnant women were all near term. In eclampsia cases an endeavor was made to secure specimens immediately after a convulsion.

The technic was as follows: Lithium citrate was used as an anticoagulant.

Nonprotein *nitrogen:* Folin-Wu.

Urea nitrogen: Van Slyke and Cullen.

Chlorides: Whitehorn.

CO_2: Van Slyke.

K, Mg., and P: Modification of Briggs for colorimetric estimation.

Na: Kramer and Tisdale, and Kramer and Gittelman.

Ca: Briggs modification of Kramer's method.

Iron: Wong.

Results: In normal pregnancy, except in the CO_2 combining power, the changes were very slight. In general there was a tendency toward lower values in NPN and urea N and a decrease in the CO_2 combining power.

There is a very slight tendency toward a lowering of the calcium in normal pregnancy, so slight as to be negligible.

Differentiation between nephritic toxemia and eclampsia was not possible by the blood chemistry determinations.

The authors use the formula $\frac{\text{B.U.N.}}{\text{N.P.N.}}$ to express the ratio of the urea nitrogen as a percentage of the nonprotein nitrogen and the formula $\frac{\text{B.U.N.}}{\text{U. N. \%}}$ to express the urea nitrogen as a percentage of the urea nitrogen in the urine.

In preeclamptic toxemia the ammonia nitrogen of the urine and the blood uric acid were slightly elevated and both ratios were lower than normal. The ratios indicated above, therefore, are of some value in differentiating between the nephritic and preeclamptic toxemias.

The most marked variations from normal were found in eclampsia. Ammonia nitrogen in the urine and the blood uric acid showed definite variations, and the CO_2 level was very low in most cases when coma intervened. The most significant changes were in the blood sugar (increased) and the inorganic P values (3.5 as compared to 2.7 mg. normal).

$\frac{\text{(P)}}{\text{(Ca)}}$ was increased: 32.36 (normal 27.83).

The hyperglycemia is assumed to be due to alterations in the liver cells.

The results are thus summarized:

1. Normal pregnancy: NPN and urea N increased; CO_2 decreased; inorganic elements within normal limits.

2. Nephritic toxemia: Increase of both the ratios noted above; inorganic elements within normal limits.

3. Preeclamptic toxemias: no outstanding variations except slight decrease in the ratios described.

4. *Eclampsia:* *uric acid increased; CO_2 decreased in proportion to intensity of coma;* blood sugar increased; increase in the P-Ca ratio which was due to increase of inorganic phosphorus.

Davidson, E. C., and Allen, C. I.: The Blood Glucose Curve in Head Injuries. Bull. Johns Hopkins Hosp., October, 1925, xxxvii, No. 4, p. 217.

It has long been known that injury to the central nervous system may be a cause of glycosuria, and the fact that glycosuria occurs, in a small percentage of cases, after serious head injuries, led Davidson and Allen to study the sugar tolerance in a series of head injuries as soon after the injury as possible and again during convalescence with a view to determining if such studies might serve as an index of the extent and severity of the damage done to the brain tissue.

Tests were made approximately sixteen hours after food was ingested and within twenty-four to forty-eight hours after the injury. In patients who recovered and in whom glucose utilization was delayed, the tests were repeated from a few days to several months later.

Due precautions were taken to minimize the effects of muscular activity and extraneous nervous influences.

Glucose was administered intravenously, 100 c.c. of a 25 per cent solution, taking ten minutes to complete the injection, and blood sugar determinations were made at 15, 30, 45, 60, 90, and 150 minutes.

No fluid was permitted during the observation period. An effort was made to secure synchronous urine samples. The spinal fluid pressure was taken six to ten hours after the test.

Studies were made of twelve concussion cases. The average fasting blood sugar was 82.6 mg., approximately normal. After the ingestion of the sugar, the curve showed a much more gradual fall than normal and the values remained above the fasting level after 90 minutes.

There was no constant relation between the blood sugar disturbance and the spinal fluid pressure.

In eighteen cases of fractured skull the findings were similar but more marked.

The data presented indicate that in serious head injuries there is a profound though temporary derangement of the carbohydrate metabolism. Observations during convalescence showed no significant variations from the normal.

The procedure does not promise much in the way of differential diagnosis. The possible mechanism is discussed.

Kubie, L. S., and Shults, G. M.: Studies on the Relationship of the Chemical Constituents of Blood and Cerebrospinal Fluid. Jour. Exper. Med., Oct. 1, 1925, xlii, No. 4, p. 565.

The paper is divided into two parts, the first concerned with studies made upon patients; the second reporting a continuation of the studies upon dogs.

In the human beings blood and spinal fluid specimens were simultaneously collected after a twelve hour fast and chemical analyses made for reducing substances (Folin and Wu), for chlorides (Whitehorn), and in some cases, for nonprotein nitrogenous constituents (Folin).

Thirty cases representing various conditions in which spinal punctures were done as diagnostic or therapeutic measures were thus studied.

The ratio of spinal fluid sugar to blood sugar varied from 0.33 to 0.81, only those fluids which contained large amounts of blood falling below 0.53. There was no difference between normal and pathologic fluids. There were no cases of acute or tuberculous meningitis or of encephalitis in the series.

No dependence of spinal fluid upon blood sugar was demonstrated, probably because the blood sugar undergoes rapid fluctuations while the spinal fluid sugar is formed and absorbed slowly.

There was no correlation between the spinal fluid sugar and the diseases encountered.

The spinal fluid chloride content is normally higher than the blood chloride content and the determinations seemed to indicate that the higher the blood chlorides, the lower is the ratio between the blood and the spinal fluid. No explanation of this is apparent.

Except in one case, the nonprotein nitrogen was lower in the spinal fluid than in the blood. This case was one of cerebral hemorrhage, and it is suggested that the concomitant low sugar is due to the action of enzymes which arose from inflammatory cellular reaction and which, through proteolysis, may also have been responsible for the nonprotein nitrogen content.

The ratio of spinal fluid to blood was from 0.51 to 0.85 for whole blood and 0.65 to 0.92 for plasma. The fluid level does not depend upon the blood level.

In the animal experiments the findings, in a general way, were comparable.

The general conclusions were that, if due allowance is made for the slow elaboration of spinal fluid and the time required for newformed fluid to emerge from a needle in the cisterna, the amounts of sugar, chlorides, and nonprotein nitrogen in the fluid in a general way follow their concentration in the blood serum. It was evident, however, that for any substance, other factors than its concentration play a part in its distribution between the blood and spinal fluid.

Rich, A. R., and Rienhoff, Jr., W. F.: Bile-Pigment Content of the Splenic Vein. Bull. Johns Hopkins Hosp., June, 1925, xxxvi, No. 6, p. 431.

Determinations were made on specimens obtained at operation and at autopsy using the van den Bergh indirect method.

In four of ten cases the splenic vein blood contained more bilirubin than blood from the splenic artery and peripheral veins, thus evidencing the formation of bilirubin in the spleen.

In pernicious anemia and secondary anemia the bilirubin content of the splenic vein may or may not be higher.

In a case of hemolytic jaundice a preoperative high bilirubin content fell to normal within four hours after splenectomy.

McCordock, H. A.: A Bacillus of the Paratyphoid Group. Bull. Johns Hopkins Hosp., December, 1925, xxxvii, No. 6, p. 412.

A cultural and biologic study of a new organism is reported in an article illustrated with microphotographs. The bacillus was isolated from the spontaneous infectious otitis seen in albino rats and the name Salmonella muriotitis is proposed.

It closely resembles the organisms of the paratyphoid group from which it is differentiated by its inability to ferment rhamnose within twenty-four hours and the absence or very slight production of gas in all carbohydrates except xylose.

Smetana, H.: Experimental Study of Amyloid Formation. Bull. Johns Hopkins Hosp., December, 1925, xxxvii, No. 6, p. 383.

The daily intramuscular injection of 0.4 to 0.6 c.c. of a sterile 5 per cent solution of nutrose produces amyloid in white mice and renders the experimental study of this substance possible.

Amyloid appears in all the parenchymatous organs. The author considers the method of Bennhold the best for the demonstration of amyloid in sections: Apply 1 per cent congo red stain with heat, wash, place in saturated lithium carbonate solution for twenty seconds, and differentiate in 80 per cent alcohol.

This can be applied to paraffin sections which can be cleared. Hematoxylin may be used for nuclei.

Zenker fixation is better than formalin, and the best results follow a twenty-four-hour fixation in cold saturated sublimate solution.

Severe damage to the cells of the parenchyma, infiltration of the cells in the periportal regions and in the capillaries of the liver, increase in the cells of the malpighian bodies of

the spleen and cell infiltration about them are constantly seen before the appearance of amyloid and during its production.

In the areas where amyloid appears there is destruction of connective tissue and elastic fibrils, swelling of the cells of the vessel walls, and dissolution of cell nuclei and fusion into a formless mass in which the first traces of amyloid appear.

Bacterial cooperation in the formation of amyloid is not necessary. The article is illustrated with colored plates.

Starobinsky, A.: The Colloidal Benzoin Reaction Applied to Syphilitic Serum. Presse méd., Paris, 1925, xxxiii, 334.

The antigen extract is prepared as follows:

Fresh horse heart muscle is finely ground, spread in glass plates and dried, first at room temperature and then in the incubator at 37° C. for sufficient time to evaporate completely all moisture. The dried mass is then finely powdered. To one part of this powder 5 parts of ether are added, and the mass extracted for forty-eight hours. It is then filtered, and the heart powder is dried in the incubator until there is no odor of ether.

To the dried mass absolute alcohol is added in the proportion of 1:9, and the powder is extracted in the incubator for 3 days. The filtrate constitutes the stock antigen extract.

To 10 c.c. of this stock extract 15 c.c. of absolute alcohol and 4 c.c. of a 10 per cent solution of benzoin resin are added. The resultant mixture should be clear and transparent.

For the test this solution is further diluted: to 1 c.c. of the antigen extract 10 c.c. of 3 per cent sodium chloride solution is added, the tube shaken, and heated to 45° C.

The test is performed by adding 1 c.c. of this final antigen dilution to 0.4 c.c. of serum inactivated for thirty minutes at 56° C.

Readings are made of the degree of flocculation at twenty-four and forty-eight hours. In the author's studies there was an agreement of 93.7 per cent between this flocculation reaction and the Wassermann.

Jordan, E. O.: The Interconvertibility of "Rough" and "Smooth" Bacterial Types. Jour. Am. Med. Assn., Jan. 16, 1926, lxxxvi, 177.

In a number of bacterial groups two types of cells occur in stock cultures, which produce, on agar plates, two types of colonies: "rough" (*R*), and "smooth" (*S*). Under ordinary conditions *S* and *R* colonies breed true. The two types are correlated with other characteristics: *S* cells form stable suspensions; *R* tend toward spontaneous agglutination. The *S* type is more virulent; acid agglutination optimum is higher for *S* than *R*, and there are serologic differences.

The *R* type predominates in old stock cultures which have been allowed to become partially dry.

Various observers have regarded *S* types as the normal, and *R* as degenerative forms. De Kruif regards $S \longrightarrow R$ transformations as a mutation.

Jordan studied 100 stock cultures of paratyphoid bacilli and demonstrated by single-cell isolations (Barber method) that either type of colony may be produced at will from a single cell.

Frequent transfers in veal infusion broth led to the *S* type, while keeping the culture in the same medium without transfer for some time led the production of *R* types. All the coincident characteristics noted above were likewise converted.

Regan, J. C. and Tolstoouhov, A. V.: *Characteristic Changes in the Blood in Whooping Cough.* Jour. Am. Med. Assn., Jan. 16, 1926, lxxxvi, 181.

Two hundred blood chemical analyses were made in cases of whooping cough to *determine if any changes of significance occurred in the blood chemistry.*

The PH (89 determinations by electrometric method) was definitely changed, ranging from 7.05 to 7.25, and values as low as 7.01 to 6.98 were occasionally encountered.

The authors feel that there is a decided relationship between the stage and severity of the disease and the PH of the blood serum, the latter being lowered in the severe cases and in the paroxysmal stage.

peroxide and extracted three times with 15 to 20 c.c. of ethyl acetate. The extract is again heated with 5 to 6 grams of anhydrous sodium sulphate and filtered through a dry paper into a weighted 100 c.c. beaker containing a few pebbles. It is evaporated to dryness on a steam bath, and then dried to constant weight at 60 to 70° C. The residue so obtained is a white crystalline solid which after weighing can be identified in the usual way.

II. *Organs.*—Weighed portions of the organs (100 to 500 grams) are minced as finely as possible and transferred to an appropriate Erlenmeyer flask containing 200 c.c. of 95 per cent ethyl alcohol and 30 c.c. of 5 per cent sulphuric acid. They are immersed in a steam bath and boiled with frequent shaking for forty-five to sixty minutes; then filtered hot onto a suction funnel, the juices being pressed out from the residue as completely as possible. The residue is transferred to a mortar and ground with 200 c.c. of ethyl alcohol, and again transferred to the Erlenmeyer flask and boiled for thirty to forty minutes. It is filtered hot, as previously described. The extraction is repeated at least twice or more, depending upon the amounts and fineness of division of the tissues. The alcoholic extracts are combined and, after cooling, are filtered by suction. They are concentrated on a steam bath until all the alcohol has been removed. The solution should be made up to about 150 c.c. with distilled water, 20 per cent lead acetate being added until no further precipitation occurs, and then treated with procedure followed in *Urine.*

With the above procedure 98 to 100 per cent recoveries of veronal from urine, and 92 to 98 per cent recoveries from organs, were obtained.

Most of the veronal is found, as a rule, in the urine, the kidney and brain containing the next largest amounts.

University of Bordeaux—A Special Course

A special course of five weeks' duration, beginning July 8, 1926, will be given by Professor Georges Portmann at the University of Bordeaux, Post Graduate School of Oto-Rhino-Laryngological Department. This course is pronounced as the best course on the continent.

Gross and minute anatomy and physiology of the ear, nose, throat, larynx and neck will be taken up in detail. Instruction will also be given in bronchoscopy, esophagoscopy and direct laryngoscopy, as well as in special treatments in oto-rhino-laryngology.

The class will be limited to twelve students and enrollment will close June 1, 1926. Fee for the course is $200.00.

For further information, apply to Dr. Leon Felderman, 4428 York Road, Philadelphia, Pa.

BOOK REVIEWS

(Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.)

*The Diabetic Diet**

AMONG the diabetic manuals this will find a definite place. That it was developed in Toronto and has the endorsement of Banting and Gilchrist should be sufficient recommendation.

There are on the market several manuals for the use of diabetic patients, varying from the rather detailed work of Joslin, in the reading of which the patient becomes thoroughly acquainted with his condition and must learn to calculate his diet with exactitude, down to those in which just sufficient information is given to enable the average diabetic to understand the basic principles of his disease and its treatment, and in which cut and dried diets are incorporated. While the authors describe them as sample menus to be altered for the individual case, we will venture to say that most patients and a large proportion of physicians base their entire treatment upon these unaltered groups of sample menus.

The reviewer feels that the most useful diabetic manual will be one with sufficient "sample menus" to cover the usual range of necessary diets, and in which provision is made for easy substitution without vitiation of the food values. In other words, skeleton diets should be provided, onto which may be built other foods in the proper proportion, selected from simple food tables.

This manual provides skeleton diets, and provides tables for the variation of vegetables and fruits. Variation of other foods such as cereals, meats, etc., is not easy. We should like to see a greater number of skeleton diets. Perhaps the best dietary schedule so far proposed for general use is that found in Mosenthal's article in Tice's "Practice." The volume under review is as practicable as any of the diabetic manuals available, however, and better than some.

Methods in Surgery†

THIS appears as a companion piece to Herrmann's *Methods in Medicine.* Both of them deal with the hospital and staff routine of the Barnes Hospital and other institutions in St. Louis, Mo. The book may be roughly divided into three sections. The first and largest contains tabloid directions for general and special surgical examinations, hospital directions for preoperative and postoperative care, and hospital rules and regulations. The second por-

*Diabetic Diet, a handbook for diabetics. By A. Doris McHenry, B.A., and Marjorie M. Cooper, B.A. with preface by J. A. Gilchrist and F. G. Banting. Cloth. Pp. 70. Harper & Brothers, New York City. 1925.

†Methods in Surgery. By Glover H. Copher, M.D., Instructor in Surgery, Washington University School of Medicine, Clinical Assistant to Barnes Hospital, Surgeon to out-patient, Washington University Dispensary, visiting surgeon, St. Louis City Hospital. Cloth. Pp. 232. Price $3.00. C. V. Mosby Co., St. Louis, 1925.

tion, contributed by Marjorie H. Copher, contains routine and special diets used on the surgical service. The last portion, which is in the nature of an appendix, presents all the special charts used on the surgical service.

The volume will be of particular interest to any who wish to work up efficient and satisfactory hospital routines and regulations.

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*The Normal Diet**

A SMALL brochure containing in very readable form the meat of a series of lectures given by the author to certain groups of patients, in which he stresses the normal metabolic requirements of the body. He presents the subject in such a manner as to be easily comprehended by the average layman, so that the latter may carry away with him an accurate knowledge of how to apply the principles of human nutrition.

Empyema Thoracis†

THIS monograph is the Samuel D. Gross prize essay accepted by the Philadelphia Academy of Surgery. In it the author discusses the newer principles of the surgical treatment of empyema that have developed since our experience with the disease during the World War. The cardinal principles emphasized by the author are: (1) careful avoidance of open pneumothorax in the acute stage; (2) the prevention of a chronic empyema by the rapid sterilization and obliteration of the infected cavity; and (3) careful attention to the nutrition of the patient. He deals with general principles rather than details and incorporates extensive discussions of the pathology, and of results with experimental empyema and pneumonia. He prefers repeated aspirations during the acute stage to continual drainage with negative pressure and recommends the Carrel-Dakin solution for sterilization and obliteration of the cavity. In a rather long addendum he surveys all criticisms of his principles, which have been made since the first presentation of his paper.

This monograph, together with Vol. II, part 2, of the report of the Medical Department of the U. S. A. in the World War, forms a very complete reference library on surgical treatment of empyema.

The Nursing of Eye Cases‡

A SMALL brochure for the use of nurses, describing the anatomy of the eye, particularly the external anatomy, and its appearance in the various common eye diseases and injuries. The author does not stress so much what is to be done in the individual case as how it should be done after the physician has so ordered.

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*The Normal Diet. By W. D. Sansum, M.S., M.D., Director of the Potter Metabolic Clinic, Dept. of Metabolism, Santa Barbara, Calif. Cloth. Illustrated. Pp. 72. Price $1.50. The C. V. Mosby Co., St. Louis, Mo., 1925.

†Some Fundamental Considerations in the Treatment of Empyema Thoracis. By Evarts A. Graham, A.B., M.D. Cloth. Illustrated. Pp. 110. Price $2.50. The C. V. Mosby Co., St. Louis, Mo. 1925.

‡By Louise Kingham, S.R.N., Matron of the Weymouth and Dorset County Royal Eye Infirmary. Paper. Pp. 16. Price thirty cents. Humphrey Milford. Oxford Univ. Press.

*Abdominal and Pelvic Surgery for Practitioners**

THIS is a treatise on the diagnosis of abdominal and pelvic diseases, both acute and chronic, in which the author presents systematically those guiding principles of diagnostic generalizations which have served him to excellent purpose during his career as a surgeon and which he illustrates with case reports from what appears to have been a wealth of clinical material.

The author divides the work into three sections, abdominal emergencies, subacute abdominal diseases, and chronic abdominal diseases.

While most attention is devoted to the diagnosis of abdominal emergencies, conditions requiring immediate operation, the essential conservatism of the author is brought out at many points in the book. We feel that this adds weight to his opinions. Thus he designates when it is best not to operate in general peritonitis, even from a perforated ulcer, the patient's best chance for recovery being by avoidance of operation. He describes the large number of operations that are performed for so-called chronic appendicitis. While there is such a condition, true chronic appendicitis is almost always associated with an antecedent acute attack.

Personal and Community Health†

INCREASING interest in individual preventive medicine necessitates the development of textbooks and monographs on this subject in which is collected the literature which is now scattered through many and diverse volumes. Dr. Turner has done this very successfully. The keynote of the work is personal health and hygiene. Of necessity community health comes in for discussion together with general principles of preventive medicine, but the chief reasons for their presentation is to outline practical measures for the maintaining of personal health. Thus food control, water supply, waste disposal, public health administration, school hygiene, industrial hygiene, ventilation, heating and lighting, are as important to the health of the individual as are proper nutrition, exercise, reproduction, oral hygiene, and mental hygiene.

The first appendix contains an alphabetical list of infectious diseases with brief descriptions of the infectious agent, source of infection, mode of transmission, incubation period, and period of communicability, followed by an outline of methods of control. The second appendix discusses disinfection and the various disinfectants.

The book should find a wide market.

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*Abdominal and Pelvic Surgery for Practitioners. By Rutherford Morison, Hon.M.A. and D.C.L., Hon LL.D., M.B., F.R.C.S., (Ed. and Eng.). Cloth. Pp 212. Humphrey Milford. Oxford Univ. Press. 1925.

†Personal and Community Health. By Clair Elsmere Turner, Associate Professor of Biology and Public Health in the Mass. Institute of Technology; Associate Professor of Hygiene in the Tufts College Medical and Dental Schools; etc. Cloth. Illustrated. Pp. 426. Price $2.50. The C. V. Mosby Company, St. Louis, Mo. 1925.

The Journal of Laboratory and Clinical Medicine

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Official Organ of the American Society of Clinical Pathologists

EDITORIALS

The Choice of a Pathologist

It is very obvious to the conscientious practitioner of medicine that the day of the man who knows it all is past, and while, to a certain type of mind, there may be some satisfaction in the assumption of omniscience for the benefit of the gaping multitude, some pabulum for the vanity in the thought that "still the wonder grew that one small head should carry all he knew," the reputation so founded is built upon sand and but the wonder of an hour.

The scientific practice of medicine revolves around the formation of a diagnosis. The formation of a diagnosis, with the subsequent elaboration of an intelligent plan of treatment, evolves from the collection, analysis, and correlation of minutia, of data procured by various means and from divers sources.

It is essential, therefore, for the physician to be familiar with the means, methods, and sources for acquiring this necessary information and capable of utilizing and applying the interpreted results to the problem at hand.

It is apparent to the most casual observer that the busier the practitioner, the more often he will require a varied investigation of his cases; and the less time, to mention only one requirement, will he have available to collect at first hand the information sought. The more necessary will it become, then, for him to utilize the specialized services of skilled associates.

We may expect of him that he shall suspect the existence of a neurologic condition or the presence of a malignant neoplasm, but we cannot demand of him in all contingencies the specialized training necessary to render a possible diagnosis absolute.

It may, however, be demanded that he shall take advantage of all means available to establish or rule out a diagnostic possibility, as, for example, by consultation with a colleague of particular skill in such matters, or through utilization of the various avenues of laboratory investigation of significance, pro or con.

It may even be said, all things being equal, that one index of the thoroughness with which a diagnostic problem is studied is the degree to which the resources of consultation are utilized, the care with which every possibility is investigated.

To seek corroboration or elimination of a possible diagnosis is not a confession of ignorance but a manifestation of wisdom; to pretend knowledge in the face of doubt or to neglect a thorough study is little less than criminal.

Considering the care with which consultants are, or should be, chosen; the value justly given to skill, training, past experience, and general reputation, one is sometimes at a loss to explain the carelessness attendant upon the selection of a clinical pathologist by the average general practitioner.

He who selects his surgeon, his radiographer, with meticulous care chooses his laboratory consultant haphazard.

Perhaps this is due, in no small measure, to an ill-advised confusion of the laboratory and the pathologist, to the habit of looking upon the two as synonymous and interchangeable terms, whereas nothing could be further from the truth.

The scalpel and the surgeon or the radiographer and his apparatus are not transposable, though one is complemental to the other, and the same is true of the clinical pathologist.

The clinician should demand of the pathologist not only the training and skill required to insure accurate and reliable findings, but, in case of need, the ability based upon experience, reading, medical education and clinical training necessary to render him available as a consultant, either from the standpoint of indicating the plan of laboratory approach most likely to be informative, or of assisting in the clinical evaluation of the results obtained.

Unfortunately for the practitioner who carelessly casts his laboratory work to the winds; who chooses because it is labeled "laboratory"; because it is near by, pretentious in appearance, or persistent in self-adulation, the necessity for extensive use of laboratory methods has resulted, by what Webster has called "the fearful concatenation of circumstance," in a mushroom growth of "laboratories" not always indicative of nor accompanied by

the coincident presence of a clinical pathologist, by which term is denoted a physician of clinical training and experience devoting himself to pathology as a specialty.

All too often the "laboratories" clamoring for the physician's "business" are entirely lay owned and lay manned, seeking by an obtrusive display of glittering apparatus and the clamorous use of technical patter to "varnish nonsense with the charm of sound."

To him who, unthinking, holds the test to be the thing; who believes that technicians can be trained in a few weeks to cover the entire range of laboratory procedures, the words of Oliver Wendell Holmes may be recalled, "Knowledge, like timber, should not be much used until it is seasoned."

The practice of clinical pathology is the practice of specialized medicine, and the technician can no more replace the clinical pathologist than the nurse or the first year student can supplant the physician.

The training of technicians is restricted entirely to methods, and, just as the clinician places the responsibility for his operations only on the surgeon, so should he place the responsibility for his laboratory studies only on the clinical pathologist. And just as he chooses his surgeon on the sole basis of skill and experience, so should he demand equivalent qualifications from his laboratory consultant.

He should appreciate, also, that to the layman the laboratory is merely a business, while to the clinical pathologist it is a profession of equal dignity and learning and subject to the same ethical and professional responsibilities as any other branch of medicine.

The man who calls the laboratory on the phone to discuss a report or talk over a case should realize that he is in consultation with a professional colleague and should demand that this be the case and not find himself debating professional problems with law technicians who may later, perhaps, be "consulting" with a cultist or even a quack.

The practitioner should always be in a position to know who does his laboratory work and be fully cognizant of his training and ability.

Less than this is an injustice to the patient and an indictment of the physician. —*R. A. K.*

The Need for a Fixed Hemoglobin Standard

A HEMOGLOBIN estimation, properly made, is one of the most valuable clinical laboratory procedures. Today widely different results may be obtained by the same specimen of blood by different laboratory workers. The clinician is often at a loss to evaluate properly the hemoglobin percentage as reported to him.

Some of the confusion is due to technical errors in apparatus for hemoglobin determination. It is not uncommon to find the standard and graduated tube of a Sahli apparatus of widely different bore. Pipettes are not infrequently incorrectly calibrated. The combined error in such an apparatus checked recently was 40 per cent.

In direct hemoglobin determination, as with the Van Slyke blood gas apparatus,[1] the hemoglobin is measured in grams per 100 c.c. of blood. For clinical application this is best expressed in percentage. In the various colorimetric procedures, the hemoglobin is read off directly in percentage of a fixed standard. In any event it is necessary to know the equivalent of the 100 per cent in terms of grams per 100 c.c. of blood.

It is usually stated that 100 per cent on a hemoglobinometer should represent the average normal number of grams of hemoglobin per 100 c.c. of blood. The hemoglobin content varies with age and sex and red cell count. In calculating the color index we consider five million cells per cubic millimeter as 100 per cent cells. The most logical standard is certainly one which takes as 100 per cent the average value of hemoglobin in grams per 100 c.c. for each five million red cells.

There are wide differences in normal hemoglobin values as given by different authors and workers. Leichenstern's[2] figures for work done in 1878 are quoted in some textbooks. Williamson,[3] using the same spectro-photometric method, obtained much higher results. Haldane's standard[4] of 13.8 grams per 100 c.c. is still used by many. This represents an average of only 12 determinations with the oxygen capacity method. No one of the workers quoted has made simultaneous red cell counts, so the figures cannot be expressed on a basis of five million red cells per c. mm. Most observers agree that the Haldane standard is too low. Newcomer,[5] in his new method, uses Williamson's figures for the normal.

It is obvious that the standard figures used must be based on an accurate method which is available generally to laboratory workers. The oxygen capacity method as adapted by Van Slyke to his blood gas apparatus is certainly the method of choice. The logical standard should take as 100 per cent, the number of grams of hemoglobin per 100 c.c. of blood, in a normal individual having five million cells of normal volume and hemoglobin content per c. mm., as determined by the Van Slyke apparatus. One series of determinations on this basis has been reported.[6]

The American Association of Clinical Pathologists could render a distinct service to laboratory workers by having a committee study the hemoglobin standard question. In this way an authoritative standard could be determined for all laboratory workers to clarify the situation.

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—*R. L. H.*

DR. FREDERIC E. SONDERN
New York City, N. Y.
President, American Society of Clinical Pathologists, 1925-1926

The American Society of Clinical Pathologists

On to Dallas—to the Fifth Annual Meeting

American Society of Clinical Pathologists

THE Fifth Annual Meeting of the American Society of Clinical Pathologists will be held in Dallas, Texas, April 15, 16, and 17, 1926, at the Baker Hotel.

The members are looking forward to this annual event with a great deal of interest and pleasure, not alone on account of its scientific value but also because of the friendships renewed and new and pleasant acquaintances formed at these reunions. It is a great stimulus to meet clinical pathologists from the larger and smaller medical centers and exchange views. The meeting forms an incentive to better work; it tends to rouse us from apathy and incites us to research and progress.

A number of important problems are to be discussed at the meeting aside from the scientific papers which in themselves offer a variety of demonstrations of interest to our specialty. A proposition that will be discussed relates to the question of standardization of laboratories or, as we prefer to term it, the improvement of the scientific status of the clinical pathologist. Considerable progress has been made in this direction by surveys instituted by our Society and other organizations, the results of which will be presented at the business session of our annual convention. Another subject of paramount importance is the valuable suggestion of Past-President John A. Kolmer of an official text of approved laboratory methods in clinical pathology. In accordance with this proposal committees will be selected from the membership to study the value of various methods and they will report it subsequently as a guide for the editors of this digest.

Questions of economic import will also have a place on the program. The spector of state medicine now looming up strongly before the medical profes-

DR. WILLIAM G. EXTON
Newark, N. J.
President-Elect

DR. JOHN A. KOLMER
Philadelphia, Pa.
President 1924-1925

DR. F. L. BURNETT
Boston, Mass.
Vice-President

DR. WARD BURDICK
Denver, Colorado
Secretary-Treasurer

sion and threatening our specialty more than any other is a problem to be reckoned with and which will be given due place in the discussion. The relation of public health laboratories to private practitioners in clinical pathology will be considered by members from the respective groups.

Other questions to be taken up will be the matter of an official publication; the awarding of a prize to stimulate original research and similar matters of interest.

Those who were privileged to be present at the Philadelphia convention will always remember with pleasure the valuable information and the intellectual treat they received during the proceedings. We anticipate a similar if not a greater thrill at the coming Dallas meeting.

All preparations that our Southern members have made presage a wonderful and pleasant gathering. In short a very lively time is anticipated for the next convention.

PROGRAM

American Society of Clinical Pathologists

Baker Hotel, Dallas, Texas

April 15, 16, 17, 1926

Officers

Dr. Frederic E. Sondern, President
New York, N. Y.

Dr. William G. Exton, President-Elect
Newark, N. J.

Dr. F. L. Burnett, Vice-President
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Dr. John A. Kolmer, Philadelphia, Pa., 1924-1925.

DR. PHILIP HILLKOWITZ
Denver, Colorado
Member of Executive Committee
President 1922-1923

DR. WM. CARPENTER MACCARTY
Rochester, Minnesota
President 1923-1924

DR. HERMAN SPITZ
Nashville, Tenn.
Chairman of Executive Committee

DR. A. H. SANFORD
Rochester, Minnesota
Member of Executive Committee

DR. THADDEUS WALKER
Detroit, Michigan
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DR A. H. SCHADE
Toledo, Ohio
Chairman of Board of Censors

DR. GEORGE IVES
St. Louis, Missouri
Member of Board of Censors

DR. ERNST A. VICTORS
San Francisco, Calif.
Member of Board of Censors

A familiar snapshot of President-Elect Wm. G. Exton, and Dr. Herman Spitz, Chairman of the Executive Committee on the Boardwalk at Atlantic City.

THURSDAY, APRIL 15, 9 A.M.

Call to Order—Appointment of Committees

Scientific Program

Hemoglobin and Erythrocytes in the South.

By Dr. Leon S. Lippincott, Vicksburg, Mississippi.

A Combined Diluting and Staining Fluid for Differential Leucocyte Count in the Counting Chamber.

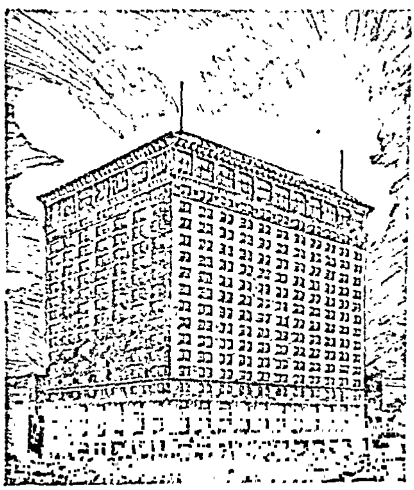
By Dr. Daniel Nicholson, Winnipeg, Canada.

Sickle Cell Anemia.

By Dr. G. S. Graham, Birmingham, Alabama.

A Photographic Method for Counting Blood Cells.

By Dr. A. H. Sanford, Rochester, Minnesota.



The Baker Hotel
Headquarters, American Society of Clinical Pathologists, April 15, 16, and 17, 1926.

THURSDAY, APRIL 15, 2 P.M.

Determination of Sugar in Normal Urine.

By Dr. Mark R. Everett, Norman, Oklahoma.

The Glucose Tolerance Test.

By Dr. W. B. Lewis, Battle Creek, Michigan.

A Study of the Pigment in Addison's Disease.

By Dr. Carl L. Spohr, and Dr. Robert A. Moore, Columbus, Ohio.

Ochronosis.

By Dr. Ernest Scott and Dr. Robert A. Moore, Columbus, Ohio.

The Treatment of One Hundred and Twenty-Five Cases of Acid Intoxication with the Buffer Solutions.

By Dr. F. A. Hecker, Ottumwa, Ia.

Clinical Results with Pathogen.

By Dr. Otto Lowy, Newark, N. J.

FRIDAY, APRIL 16, 9 A.M.

Frozen Sections, Their Place, Value, and Methods.
By Dr. L. A. Turley, Norman, Oklahoma.

Laboratory Examinations Necessary and Unnecessary.
By Dr. George L. Schadt, Springfield, Massachusetts.

The Integration of Hospital Laboratory Work.
By Dr. Philip Hillkowitz, Denver, Colorado.

The Cytomorphosis of the Tubercle Bacillus.
By Dr. Harry J. Corper, Denver, Colorado.

Oxygentherapy.
By Dr. Paul Roth, Battle Creek, Michigan.

A Key to the Diagnosis of Neoplastic Conditions.
By Dr. William Carpenter MacCarty, Rochester, Minnesota.

FRIDAY, APRIL 16, 2 P.M.

Intestinal Amebiasis from the Pathological Standpoint as Related to the Clinical, with Preliminary Report of X-Ray Studies of Early Cases.
By Dr. J. M. Feder, Panama.

Treponematosis as Seen in the Rural Population of Haiti.
By Dr. C. S. Butler, Port-au-Prince, Haiti.

The Hirsch-Abderhalden Test.
By Dr. F. E. Sondern, New York City.

Comparison of Kolmer and Kahn Tests.
By Dr. C. E. Roderick, Battle Creek, Michigan.

Comparative Result with the Vernes and Wassermann Tests.
By Dr. L. H. Cornwall, New York City.

FRIDAY, APRIL 16, 7 P.M.

Annual Dinner at the Baker Hotel

Presidential Address.
By Dr. Frederic E. Sondern, New York City.

Greetings from the American College of Surgeons.
By Dr. M. T. MacEachern, Chicago, Illinois.

Welcome to Dallas.
By Dr. Edward F. Cooke, Houston, Texas.

SATURDAY, APRIL 17, 9 A.M.

Business Session

What Has Been Done in the Last Year by the American Medical Association in Regard to Standardization of Laboratories.
By Dr. N. P. Colwell, Chicago, Illinois.

SATURDAY, APRIL 17, 2 P.M.

Business Session

Commercial Exhibits

Laboratory workers, more than most any other group of physicians, need to keep in close touch with the manufacturers of the supplies and equipment which they need to carry on their work successfully.

The Executive Committee of the American Society of Clinical Pathologists decided in 1925 that the members and visitors at the annual meeting of the Society would derive a great deal of good from a commercial exhibit by the manufacturers who make and sell the articles used in the medical laboratory.

Such an exhibit was held in conjunction with the Philadelphia Meeting, and it proved to be an outstanding success both for the members and the exhibitors. Such an exhibit will be held at the Dallas Meeting, and members of the Society and their friends attending the meeting will be given an opportunity to meet representatives of the firms from whom they purchase their supplies. Such a meeting is always mutually profitable. Firms which supply laboratories with the essential materials and equipment to carry on successfully are rendering no less a service to medicine and humanity than are the laboratory workers themselves.

There would be no laboratories without instruments of precision, reagents, and stains. If this exhibit succeeds in establishing a closer working relationship between the laboratory and the firms making the supplies and equipment, then everyone connected with it will be content. Dr. L. A. Duck will be in charge of the exhibits.

The Journal of Laboratory and Clinical Medicine

Vol. XI St. Louis, Mo., May, 1926 No. 8

CLINICAL AND EXPERIMENTAL

EXSANGUINATION-TRANSFUSION IN THE TREATMENT OF MERCURIC CHLORIDE POISONING*

By Chas. C. Haskell, J. R. Hamilton, and W. C. Henderson,
Richmond, Va.

BURMEISTER and McNally[1] have shown that the absorption of mercuric chloride from the alimentary canal occurs with great rapidity; within three minutes after the oral administration of a massive dose of the poison, sufficient absorption has occurred to produce "irrecoverable damage" to the kidney. It is an unfortunate fact that many patients suffering from poisoning by mercuric chloride are not subjected to treatment for some little time after ingestion of the mercury. It is to be assumed, therefore, that in these cases considerable absorption has taken place before the employment of measures designed to remove the mercury from the alimentary canal; furthermore, it is permissible to assume that the longer the metal remains in the blood, the greater the chance of injury to the kidneys and other vital organs. The number of chemical antidotes that have been proposed for mercuric chloride bears testimony to the universal recognition of these facts; their abandonment by the majority of clinicians, together with adverse experimental evidence that has been obtained in regard to certain of them, bears testimony to their practical uselessness.

In a recent paper, Robertson[2] has proposed a method, which he designates as "exsanguination-transfusion," for the treatment of certain forms of intoxication. While Robertson recommends this procedure especially in cases suffering from poisoning by bacterial products, nevertheless he is inclined to the belief that it may be of value in dealing with other forms of poisoning;

*From the Department of Pharmacology, Medical College of Virginia, Richmond, Va.
Received for publication, July 25, 1925.

indeed, instances are cited where it appeared to act beneficially in poisoning by resorcinol. Rosenbloom[3] has shown that a relatively large amount of the mercury is still present in the blood as long as ten days after administration of a fatal dose. It would seem, therefore, that Robertson's method of treatment should be especially efficacious in poisoning by mercuric chloride. Even before the appearance of Robertson's paper, Burmeister[4] had made the suggestion that removal of a large part of the patient's blood and its substitution by an unpoisoned blood might be of benefit in mercury poisoning. As judged by the survival of the animals used by this author, the method is of little value; the fact that certain of his treated animals appeared to suffer less renal damage than did the controls offered him some encouragement. Burmeister's technic differed from that proposed by Robertson; moreover, considerable time elapsed between the subcutaneous injection of the mercuric chloride and the commencement of treatment: these facts appeared to justify further experimentation along similar lines.

Dogs were used exclusively in the present study. The donors, apparently healthy, adult animals, were etherized and the femoral artery exposed under aseptic precautions. A glass cannula was inserted into this vessel and the blood allowed to flow into a sterile flask, containing a sufficient amount of a 3.5 per cent solution of sodium citrate to make a final concentration of this salt of 0.35 per cent. In all but two instances, the blood was used within two hours after securing it from the donor; in these two cases, the blood was preserved in the ice box for about twenty-four hours. By a somewhat crude method, the blood of donor and recipient was matched in all cases, no instances of incompatibility being seen.

Because of its greater accuracy, the intravenous administration of the mercuric chloride was employed. Sansum[5] has shown that by this method of administration, the minimal fatal dose of mercuric chloride for dogs may be determined with great accuracy. In a large number of animals, our experience has been so in accord with his that failure of any adult dog, regardless of weight, to succumb following the intravenous injection of 4 mg. mercuric chloride per kg. body weight arouses the suspicion of error in dosage or that a portion of the fluid had escaped outside the vessel, an accident which occurred in one of the control dogs of our present series.

Six dogs were used as controls for the mercuric chloride. In three of these, under morphine-procaine anesthesia, the femoral vein was exposed, and the mercuric chloride injected from a Luer syringe, a 1 per cent solution in distilled water being employed. In two other dogs, the injections were made into the jugular vein without dissection or anesthesia; in a sixth dog, one-half the dose (2 mg. per kg.) was injected into the jugular vein as described; the following day, the remainder was injected into the exposed femoral vein. As has already been mentioned, in the case of one dog, No. 9, we had reason to believe that the needle penetrated the vein wall and that a portion of the calculated dose escaped into the perivascular tissues. As may be seen from Table I, all the animals save this one succumbed to the effects of the mercury.

TABLE I

MERCURIC CHLORIDE, 4 MG. PER KG. INTRAVENOUSLY

NO.	WEIGHT IN KG.	INJECTION SITE	RESULT	REMARKS
5	11.0	Femoral	Died in 8 days	
9	5.3	*Femoral*	*Recovered*	*Injection probably extravascular*
12	6.5	Femoral	Died in 2 days	
16	2.3	Jugular	Died in 15 hours	
17	4.1	Jugular	Died in 9 days	
18	8.3	Jugular-Femoral	Died in 3 days	

After receiving an intravenous injection of mercuric chloride, 4 mg. per kg., six dogs were bled and subsequently transfused with whole blood. The procedure followed in five of these animals is illustrated by the following protocol:

March 13, 1925.

Dog No. 1. *Brown female, weight 6.7 kg.*

2:41 P.M. Morphine sulphate, 20 mg. per kilogram subcutaneously.

3:45 P.M. Under the morphine-procaine anesthesia, the femoral vessels have been exposed; 4 mg. mercuric chloride injected into femoral vein from Luer syringe (1 per cent solution).

3:59 P.M. Three hundred c.c. of blood withdrawn from femoral artery, followed immediately by injection of 350 c.c. of blood from donor into femoral vein.

4:10 P.M. Two hundred c.c. of blood withdrawn from artery; followed immediately by injection of 250 c.c. of blood into vein.

Wound closed with sutures; dry, sterile dressing. At removal from table, dog seemed in fair condition, except for depression from morphine.

March 14, 1925.

7:45 A.M. Dog stiff in death.

In some of the other instances, smaller amounts of blood were removed; in others, larger quantities. Invariably, however, the procedure seemed to have a deleterious effect; in one instance, the dog succumbed on the table; in five others, death occurred in 10, 30, 30, and 46 hours, respectively, after treatment. In order to avoid the necessity of making an incision and introducing the possibility of infection, the sixth dog was treated as follows:

April 24, 1925.

Dog No. 24. White and brown male puppy; weight 5.3 kg.

2:42 P.M. Morphine sulphate, 20 mg. per kilogram subcutaneously.

3:04 P.M. Mercuric chloride, 4 mg. per kilogram, injected into jugular vein.

3:07-3:21 P.M. Three per cent of body weight in blood removed by means of a syringe from jugular vein.

3:28 P.M. Fifty c.c. fresh, mated blood withdrawn from jugular of donor and injected into jugular of recipient.

3:30 P.M. Sixty c.c. fresh blood injected into jugular.

3:32 P.M. Fifteen c.c. fresh blood injected. Respiration ceased; artificial respiration and cardiac massage employed to no avail.

The results on all six dogs are given in Table II.

Such results as these are not calculated to render one enthusiastic: not only did we fail to save any one of the treated animals, but the average duration of life for the three dogs that did not die on the table was less than twenty-four hours; while the average life of the controls, excepting one that died in fifteen hours, was over five days. The question immediately arises

TABLE II

RESULTS OF EXSANGUINATION-TRANSFUSION TREATMENT

NO.	WEIGHT IN KG.	$HgCl_2$ MG. × KG.	INTERVAL BETWEEN ADMINIS. $HgCl_2$ AND TREATMENT	AMT. BLOOD WITHDRAWN % BODY WT.	RESULT
1	6.7	4	14 minutes	5.6	Death in 10 hours
2	9.0	4	15 minutes	5.3	Death in 30 hours
3*	9.0	4	20 minutes	5.5	Death in 30 hours
21	6.8	4	23 minutes	9.0	Death on table
24†	5.3	4	3 minutes	3.0	Death on table

*Ether anesthesia.
†Transfused directly into jugular without dissection.

whether our technic of transfusion was not responsible for the unfavorable outcome; against this is the fact that, using a suspension of washed corpuscles, Burmeister, likewise, obtained discouraging results, as far as duration of life in his animals was concerned. In order to test this possibility, however, seven dogs were transfused as controls, they having received no mercury. Except for this omission, the procedure was the same as that followed in the case of the poisoned dogs receiving the exsanguination-transfusion treatment. Of these seven controls, the operative wound became badly infected in two cases, leading to the death of the animals on the fourth and the seventh days respectively; a third dog seemed to be doing well following transfusion, but suffered a fatal hemorrhage on the fifth day following the operation. The remaining four dogs seemed in no way injured by the transfusion and were in excellent condition at the end of 8, 8, 11, and 14 days, respectively, when they were used for other purposes. In no single instance did we observe the sudden death which was seen in the case of two of the mercury dogs; indeed, the deaths that occurred in these controls were only indirectly connected with the transfusion.

It may be suggested that the mercury so weakens the resistance of the animals that have received a fatal dose of it that they are unable to resist injuries which a normal dog withstands. This is possible, but it seems unlikely that weakening to any great extent would occur even in twenty-three minutes, the longest interval that elapsed between the administration of the mercuric chloride and the institution of treatment in our dogs. Whatever the explanation may be, it seems undoubtedly proved that the exsanguination-transfusion method of treating mercuric chloride poisoning is distinctly injurious.

For a number ... s, the opinion has been held by certain observers
that there is a rel... between the hydrogen-ion concentration of the
blood and the func... acity or even structural integrity of the kidney.[6]
The possible impor... his relationship has been recently stressed by
MacNider,[7] in conn... poisoning ... anium and by mercury; in
their enthusiasm, o... s have dr... clusions from MacNider's
work which are sca... ed. Thus ... pparently attaches great
importance to the a... of alkal... ously to patients suffer-
from mercuric c... ing. I... [9] also recommends the
of alkaline treatm... ases. ... pointed out[10] that the

administration of considerable amounts of sodium bicarbonate to dogs that had received a fatal dose of mercuric chloride intravenously did not appear to exert any favorable influence, as judged by the duration of the animal's life or by the degree of urea retention in the blood; it was felt, however, that the use of alkaline solution intravenously after the removal of a considerable amount of the animal's blood might prove more efficacious.

Three dogs were given 20 mg. morphine sulphate per kilogram subcutaneously; the femoral vein was exposed under procaine anesthesia, and 4 mg. mercuric chloride per kilogram body weight injected into this vessel from a Luer syringe. In from seven to fifteen minutes after injecting the mercury, the animals were bled from 3 to 3.9 per cent of their body weight. Immediately afterward, they received intravenously either 1 per cent or 5 per cent bicarbonate of soda solution. A fourth dog was given the mercury and, subsequently, the bicarbonate, in the form of a 5 per cent solution, without the preliminary bleeding. As may be seen from Table III, in no instance were we able to save the life of a dog by this procedure; death occurring in from ten hours to three days after the injection of the mercury.

TABLE III

RESULTS OF BLEEDING-BICARBONATE TREATMENT IN MERCURIC CHLORIDE POISONING

NO.	WT. KG.	$HgCl_2$ MG. × KG.	INTERVAL BETWEEN ADMINIS. $HgCl_2$ AND TREATMENT	BLOOD WITHDRAWN % OF BODY WEIGHT	PERCENTAGE SODIUM BICARBONATE	DEATH AFTER
7	11.0	4	7 minutes	3	1*	48 hrs.
11	11.9	4	15 minutes	3	1*	10 hrs.
23	19.6	4	13 minutes	3.9	5*	3 days
24	7.4	4	9 minutes	0	5†	3 days

*These dogs received slightly more of the bicarbonate solution than the volume of blood removed.

†This dog was given 20 c.c. of 5 per cent bicarbonate solution per kilogram.

CONCLUSIONS

1. The dose of 4 mg. of mercuric chloride intravenously is fatal to the great majority of dogs, unless there is reason to suspect faulty technic.

2. After the injection of 4 mg. mercuric chloride per kilogram intravenously into dogs, the removal of large amounts of blood and the subsequent injection of a compatible blood from another animal or of a solution of bicarbonate of soda, 1 to 5 per cent, appears to have not the least value, as judged by the early death of the animals treated in this manner.

REFERENCES

[1]Burmeister and McNally: Acute Mercury Poisoning, Jour. Med. Research, 1917, xxxvi, 87.
[2]Robertson: Exsanguination-Transfusion. A New Therapeutic Measure in the Treatment of Severe Toxaemias, Arch. Surg., July, 1924, ix, 1.
[3]Rosenbloom: A Note on the Distribution of Mercury in the Body in a Case of Acute Bichloride of Mercury Poisoning, Jour. Biol. Chem., 1915, xx, 123.
[4]Burmeister: The Effect of Extensive Venesection and Transfusion on Kidney Lesions in Severe Acute Mercuric Chloride Poisoning, JOUR. LAB. AND CLIN. MED., 1916 and 17, ii, 500.
[5]Sansum: The Principles of Treatment in Mercuric Chloride Poisoning, Jour. Am. Med. Assn., March 23, 1918, lxx, 824.
[6]See Goto: A Study of the Acidosis, Blood Urea, and Plasma Chlorides in Uranium Nephritis in the Dog and of the Protective Action of Sodium Bicarbonate, Jour. Exp. Med., 1917, xxv, 693, for literature.

[7]MacNider: The Inhibition of the Toxicity of Uranium Nitrate by Sodium Carbonate and the Protection of the Kidney Acutely Nephropathic from Uranium from the Toxic Action of an Anesthetic by Sodium Carbonate, Jour. Exp. Med., 1916, xxiii, 171.

A Study of Acute Mercuric Chloride Intoxications in the Dog, with Special Reference to the Kidney Injury, Jour. Exp. Med., 1918, xxvii, 519.

[8]Weiss: Mercuric Chloride Poisoning, Arch. Int. Med., 1924, xxxiii, 224.

[9]Rosenbloom: Studies in a Case of Acute Bichloride of Mercury Poisoning Treated by the Newer Methods and Followed by Recovery, Am. Jour. Med. Sc., 1919, clvii, 348.

[10]Vanderhoof and Haskell: The Relation of Acidosis to Nitrogen Retention in Experimental Nephritis, Southern Med. Jour., March, 1923, xvi, 170 to 176.

STUDIES ON NUTRITION IN TUBERCULOSIS*

II. Experimental Tuberculous Infection in the Albino Rat and the Influence of Vitamin Deficient Diets Thereon

By Maurice I. Smith, M.D., with the assistance of E. G. Hendrick, Washington, D. C.

THAT nutrition plays an important rôle in the prevention and treatment of tuberculosis has long been recognized by clinical observers. Nearly all writers on the subject of tuberculosis emphasize the importance of diet in the treatment of this disease. Statistics on malnutrition and tuberculosis prevalence seem clearly to point to an intimate relationship of cause and effect rather than of mere coincidence. Though it is generally conceded that malnutrition influences tuberculosis unfavorably and may be the cause of increased tuberculosis prevalence, the converse cannot be accepted without proof, which is still lacking. Even the generally accepted premise that malnutrition affects tuberculosis unfavorably lacks precision of definition, for it is not known whether it is insufficiency of food in general or inadequacy of one or more of known specific dietary constituents that is the important causative factor in lowering bodily resistance.

Stimulated by the researches on the fat-soluble food accessory in its relation to calcium metabolism, some studies were undertaken several years ago on the influence of this vitamin on experimental tuberculosis of the guinea pig.[1]† Because of the technical difficulties involved in carrying out nutrition experiments upon the guinea pig, the results were not conclusive, but were sufficiently suggestive to encourage further work upon a more suitable laboratory animal. After some consideration the albino rat was selected for this purpose.

The choice of this animal was made for the following reasons: The nutritional requirements of the white rat have been thoroughly studied in recent years. This animal is omnivorous in its dietary habits, and the vari-

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*A preliminary [illegible] of this work was presented before the Pathological Society of the Feder[illegible] [illegible]ties for Experimental Biology, St. Louis, December, 1923; and before [illegible] of the National Tuberculosis Association, Atlanta, May 9, 1924.

From Division of Pharmacology, Hygienic Laboratory, Washington, D. C.

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Received for publication, August 3, 1925.

†The reader is referred to these papers for a brief survey of the literature on the subject under discussion.

ous dietary factors can be controlled more or less completely. Because of the small size of the animal and the relatively small amount of food it consumes, large numbers can be cared for in a limited space, and the feeding of synthetic diets consisting of purified foodstuffs, which would otherwise be well-nigh impossible, can be accomplished without much difficulty. The rat is very susceptible to vitamin deficiencies, especially fat-soluble vitamin *A* and water soluble vitamin *B*. Its susceptibility to tuberculosis, on the other hand, would not seem to be very great, judging from some of the reports in the literature. It seemed quite reasonable, therefore, to attempt to break down the natural resistance of this animal to tubercle infection by depriving it of some of the dietary accessories which are known to be so vital in its nutrition and metabolism.

EXPERIMENTAL TUBERCULOSIS IN THE WHITE RAT

The information gathered from the literature on experimental tubercle infection in the albino rat is not altogether satisfactory. Vagedes,[2] working apparently with human strains of tubercle bacilli, stated that intravenous injection in the rat produced a few pulmonary tubercles in two months. Aoki,[3] in 1913, tested the virulence of several human and bovine strains by intraperitoneal injection in the rat, and concluded that the human strain was more virulent than the bovine for this species. It is stated that the lungs were the seat of involvement, exclusively.

Galli-Valerio[4] describing the effects of the tubercle bacillus in the white rat states that either the human or bovine strain is slow in developing in this animal, and in either case almost always produces pulmonary lesions. Enlargement of the spleen was also noted, though bacilli, it is stated, could not be found in either the spleen or liver.

From the work of Lewis and Margot[5] on the spleen in relation to tuberculosis resistance it would appear that the bovine bacillus injected intraperitoneally in the rat produces a splenic tumor, often hemorrhagic, and that the infection runs a rather rapid septicemic course.

Boquet and Negee[6] have stated that white rats are but little susceptible to tuberculosis. It is stated that 1 to 2 mg. of human, bovine, or avian bacilli injected intraperitoneally in the albino rat provoke lesions in the lungs, with multiplication of bacilli in other organs, which, however, may not show any lesions. The animals rarely succumb to the infection.

The British Royal Commission on Tuberculosis investigated this question, and their extensive reports may be briefly summarized as follows: Rats appear to be quite refractory to subcutaneous inoculation of bovine bacilli, but are more susceptible to intraperitoneal injections, the smallest dose being about 5 mg. Following such inoculation the rat dies as a result of the multiplication of bacilli in its tissues, but there is no development of the characteristic tubercle as seen in other animals. The tubercle bacillus is intracellular, but produces little tissue destruction. Similar results were also obtained with the human strain.[7, 8, 9]

In a recent publication Gloyne and Page[10] have contributed much infor-

mation on the reaction of the albino rat to *B. tuberculosis.* A virulent human strain was used in their experiments, the inoculation being made subcutaneously or intraperitoneally. The tissue changes following such inoculation are described as consisting of collections of phagocytes containing the bacilli, found for the most part in the spleen, although some were also found in the

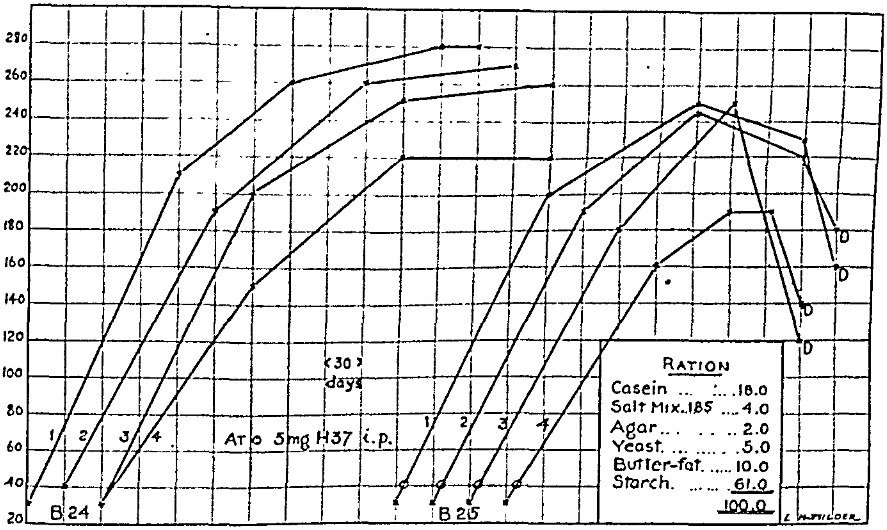


Chart. I.—Shows the course of tubercle infection in the white rat, maintained on a diet adequate in all respects. Group *B* 24 represents the weight curves of four control male rats, and group 25, of four males infected with B. tuberculosis (H 37). It will be seen that the infected animals on the adequate ration made a nearly normal growth, during the maximum growth period of about 8 months, then declined rather abruptly, death occurring about a year after infection.

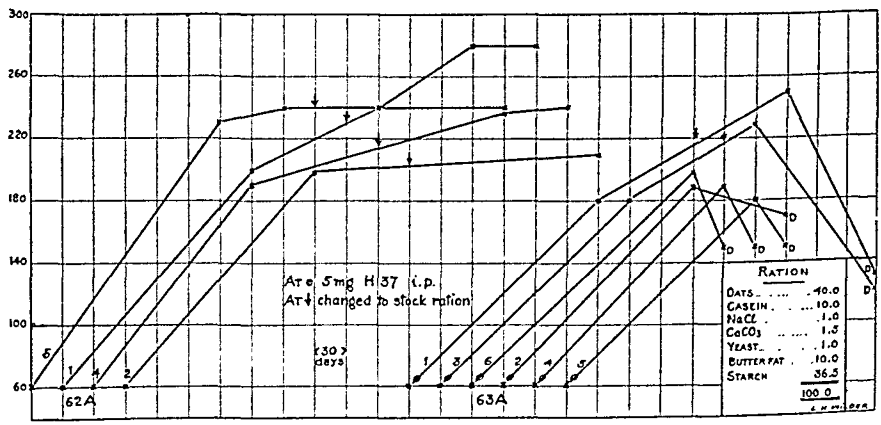


Chart II.—Course of tuberculous infection in the white rat on a diet of oats supplemented with casein, butter, yeast, and salts. Several animals in these groups developed respiratory symptoms which probably hastened death of some of the animals of the infected group. The synthetic diet was replaced by the stock ration at the point indicated in the chart. The results shown in this chart agree in the main with those of Chart I.

lungs, lymph glands, bone marrow and liver. Frequently the conglomerate masses of phagocytes presented a striking resemblance to true giant cells, though lacking the characteristic peripheral grouping of nuclei. Caseation was not noted. The masses of phagocytes are regarded by the authors as the result of endothelial proliferation, originating probably from the lymph

vessels. Curiously enough, tubercle bacilli were only occasionally found in the endothelial cells, but were most commonly seen in the "giant cells." Phagocytosis appeared to be the most marked feature in the process, and attempts were therefore made to increase the susceptibility of the rat to tubercle infection by means of various agents tending to inhibit phagocytosis. The results were negative. Experiments on sensitization of inoculated rats to tuberculin also failed to yield results. The work of these authors is referred to at some length, because the experiments described herein were carried out, at about the same time, and some of the results obtained by us are somewhat at variance with those of Gloyne and Page.

A virulent human strain, H 37 grown on glycerin agar or glycerin broth, was used in the present experiments. Emulsions of 5 mg. of moist weight per cubic centimeter in sterile physiologic salt solution were prepared, and doses of one cubic centimeter of the emulsion were injected intraperitoneally. The lesions produced by this treatment in young animals maintained on an adequate diet become clearly manifest in from thirty to sixty days, and progress till the death of the animal. The length of life in such infected animals usually runs from ten to fifteen months (see Charts I and II). Animals infected and maintained on an adequate diet, such as

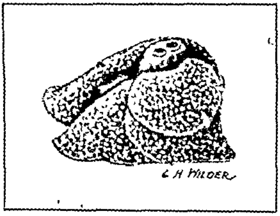


Fig. 1.—Appearance of lungs of rat infected with B. tuberculosis, human strain (H 37). Animal was killed about 6 months after infection. The lungs are voluminous, do not collapse, and are studded with grayish white nodules.

are shown in Charts I and II, show no evidence of ill health for many months, run a nearly normal weight curve throughout their growth period of about six to eight months, but soon thereafter begin to decline rather abruptly. During the period of decline the animals show some embarrassment of respiration, loss of appetite, apathy, emaciation, and a moderate degree of cyanosis. Some animals may live with such symptoms for several months and show an extreme degree of cachexia, though as a rule, when once developed these symptoms become aggravated rather rapidly, the animals appear in ill health, and die within a month or two.

The autopsy findings are emaciation and loss of subcutaneous and other body fats. The spleen is generally much enlarged and congested. The lungs are voluminous, do not collapse, and are mottled with grayish yellowish conglomerate "tubercles." (Fig. 1.) These nodules are so numerous in the advanced cases that there is hardly any normal lung tissue left. The lung

lesions are of constant occurrence and, together with the enlarged spleen, constitute the most striking feature macroscopically.

The tubercle bacillus was isolated on several occasions from the pulmonary lesions, and guinea pigs inoculated with either the culture or with the infected lung material proved the pathogenicity of the recovered microorganism.

The histologic findings are briefly as follows: As early as six days after inoculation there is some cellular proliferation in the omentum with the formation of large multinucleated eosinophilic cells presenting an appearance

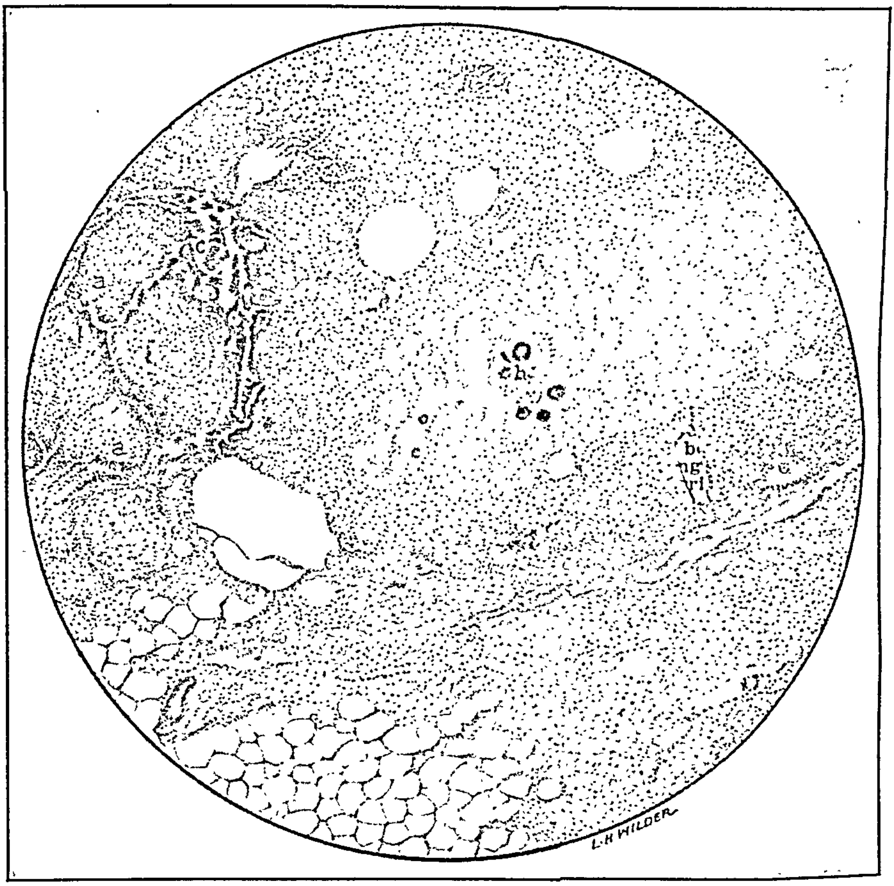


Fig. 2.—Omentum of rat infected with B. tuberculosis. Note the multinucleated eosinophilic cells forming syncytium-like areas (*a*), giant cells (*b*), and lymphocytic infiltration (*c*). 16 mm. objective, 10 eye piece.

that may be likened to a syncytium. In two weeks the proliferation of these multinucleated cells has extended to the spleen and liver. As the disease progresses these cells are very numerous in the omentum and spleen (Figs. 2 and 3), but never proliferate to any great extent in the liver (Fig. 4). In some instances a moderate degree of periportal cirrhosis may be seen as shown in Fig. 5. An occasional typical giant cell may be seen, and foci of lymphocytic infiltration is a constant occurrence in the omentum of moderately advanced infection. Proliferation of the eosinophilic multinucleated cells is also a marked feature in the lymph glands, as is shown in Fig. 6,

which represents a section of the substernal gland, taken one month after inoculation.

The pulmonary lesions are first observable histologically about one month after inoculation. The lesions are still very few at this time, but become quite numerous after sixty to ninety days following inoculation. Histologically the pulmonary tubercle is seen to consist of loosely packed aggregates of epithelioid cells, multinucleated cells resembling those seen in the lymphoid tissues referred to above, and occasionally true giant cells. Lymphocytes may be seen scattered through the "tubercle" or in discrete foci, with a tendency to arrange themselves peripherally around the large multinucleated cells. The epithelioid cells may be seen as distinct cells with

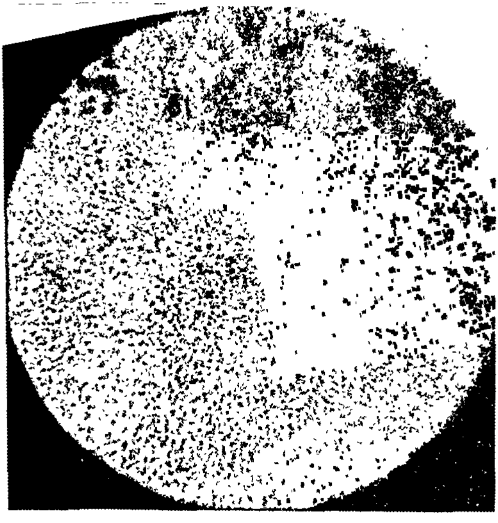


Fig. 3.—Spleen of rat infected with B. tuberculosis. Shows proliferation of multinucleated eosinophilic cells invading the Malpighian corpuscles and replacing the normal spleen pulp

Photomicrograph, 4 mm. objective, 6.4 eye piece. Hematoxylin and eosin.

definite cell outlines or closely packed together, into what appears to be a single multinucleated cell. Carbolfuchsin stained sections of tissues that had been fixed in Zenker-formalin solution show the intracellular distribution of the tubercle bacillus in the pulmonary lesions, the bacilli being present in the epithelioid, multinucleated and the giant cells. These points are illustrated by Figs. 7 to 10. Sections of lungs of animals dying of the infection show enormous cellular proliferation, there being almost complete disappearance of alveolar spaces, and the cells are literally loaded with bacilli. (See Figs. 11 and 12.)

In hematoxylin-eosin sections of Zenker-formalin fixed tissues the cytoplasm of the epithelioid and multinucleated cells comprising the pulmonary "tubercle" often presents the appearance suggestive of minute fat globules. The lipoidal nature of these cells has now been abundantly verified by staining sections of frozen fresh or formalin fixed tissues with sudan III and osmic acid. Reference to Fig. 13 will clearly show that the cell aggregates, in which bacilli are found, have undergone certain fatty changes. Applica-

Fig. 4.—Photomicrograph of the liver of a rat infected with B. tuberculosis. Note the multinucleated cell (*a*) in relation to the sublobular vein and the bile duct. Hematoxylin and eosin, 16 mm. objective, 5 eye piece.

tion of osmic acid and sudan stains indicates that the fat is unsaturated, that it is intracellular, and finely granular.

This intracellular lipoid has been found in the pulmonary "tubercle" exclusively, whenever the latter has been subjected to examination, and the indications are that it is not influenced by changes in diet or by chemical agents, though further experiments on this point are in progress. There is no information at present available as to whether this lipoid is a product of the tubercle bacillus or of the altered metabolism of the cell within which the bacillus multiplies.

The multinucleated cells of other tissues of the infected rat, such as the spleen, liver, omentum, and glands do not undergo lipoidal transformation demonstrable by staining methods hitherto applied. It is perhaps significant that the multinucleated cells of these organs harbor relatively few bacilli, as may be judged from examination of carbolfuchsin stained sections of tissues fixed in Zenker-formalin.

Following the conception developed by Evans, Bowman and Winternitz,[11] Foot,[12, 13] and others of the endothelial origin of the epithelioid cell,

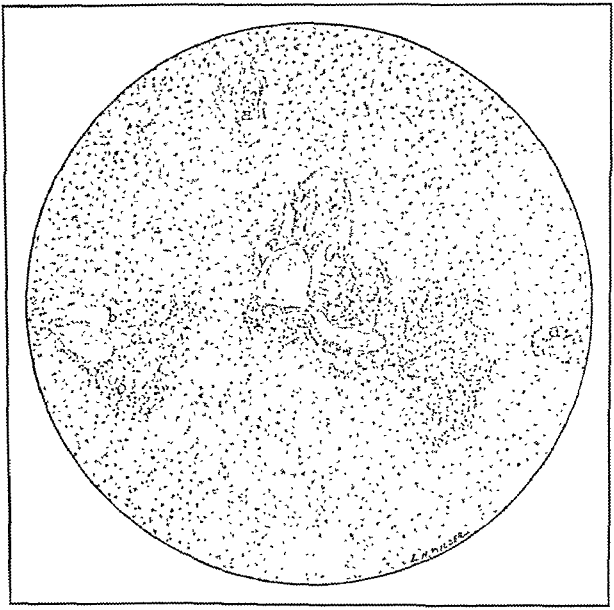


Fig. 5.—Liver of a rat infected with B. tuberculosis. Shows syncytial multinucleated eosinophilic cells within the liver lobule (*a*), in the portal canals (*b*), and some periportal cirrhosis. 16 mm. objective, 10 eye piece.

it seemed that it might be possible to influence the relatively simple cellular response to the tubercle bacillus in the rat by means of such agents as are known to be phagocyted by certain cells of endothelial origin. With this *aim in view albino rats were treated with vital dyes and also inoculated with* the tubercle bacillus. The dyes were administered either intravenously or intraperitoneally in doses of 5 to 10 mg. over considerable periods of time. Experiments with trypan-blue, which have yielded most interesting results thus far, clearly show that the vitally stained cell, probably the clasmatocyte, *which according to the recent researches of Sabin, Doan and Cunningham*[14]

is of endothelial origin, is quite distinct from the cell concerned with the reaction to the tubercle bacillus. In the spleen of the infected rat we find the large multinucleated syncytium-like eosinophilic cells, while in vitally stained nontuberculous animals there is evidence of much proliferation of megakaryocytes, and numerous clasmatocytes are found. The histologic picture of the lungs of vitally stained infected animals is most striking. The vitally stained clasmatocytes are seen in the interalveolar spaces, around the bronchioles, and in the neighborhood of the blood vessels. The epithelioid

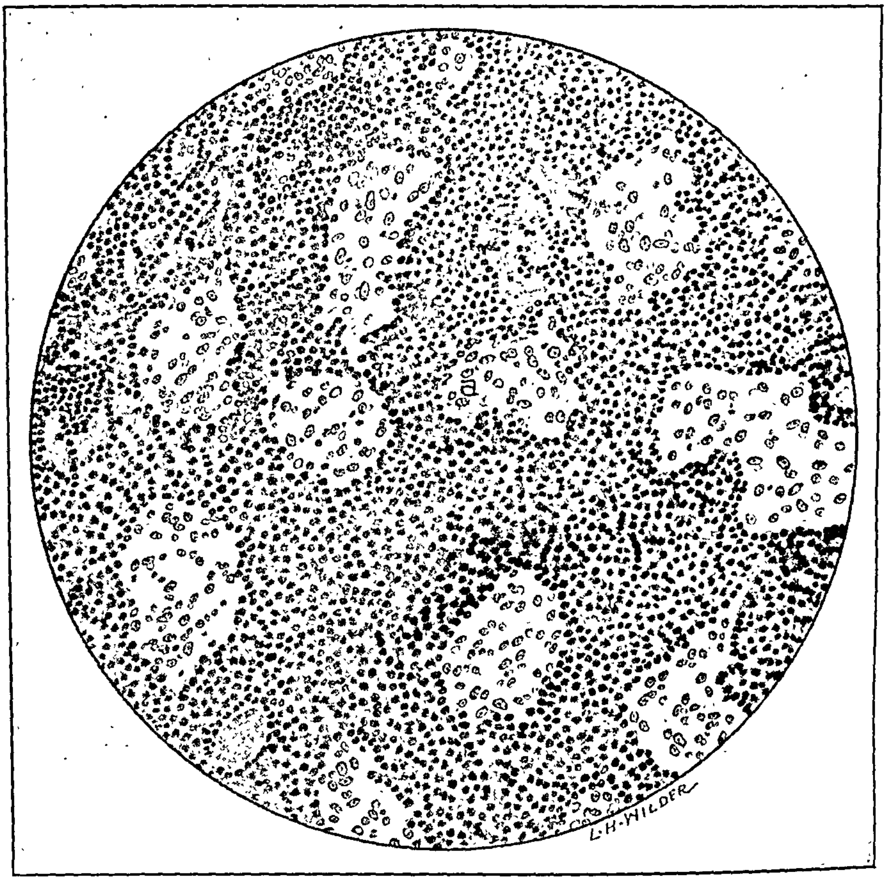


Fig. 6.—Substernal lymph gland of a rat after 30 days of inoculation with B. tuberculosis. Note the syncytial eosinophilic cells invading the lymphoid follicles. 4 mm. objective, 10 eye-piece.

and multinucleated cells of the cellular aggregate, formed in response to tubercle infection, are not vitally stained. This can be most clearly demonstrated in frozen sections of fresh lungs of animals infected and vitally stained with trypan-blue, and later stained with sudan III. The cellular aggregate, constituting the pulmonary tubercle in the rat stains more or less pink, depending on the amount of intracellular lipoid present, while the clasmatocytes are blue by contrast, showing a distribution as outlined above. By means of variously stained serial sections it can be shown clearly that the epithelioid and multinucleated cells, probably the monocytes of Sabin, Doan

and Cunningham,[14] contain the tubercle bacillus, and are chiefly concerned with the reaction thereto, while the vitally stained clasmatocyte is in no apparent relation to the tubercle. All attempts at modifying the tissue reaction of the rat to the tubercle bacillus by means of vital stains have failed to yield results, which is quite in harmony with the cited observation.

TUBERCULIN HYPERSENSITIVENESS IN RATS INFECTED WITH B. TUBERCULOSIS

This was studied in albino rats infected with 5 mg. of H 37 intraperitoneally and maintained on diets variously supplied with vitamin *A*. A single sample of a commercial old tuberculin was used in this series of experiments. The animals were tested at intervals ranging from 50 to 165 days following

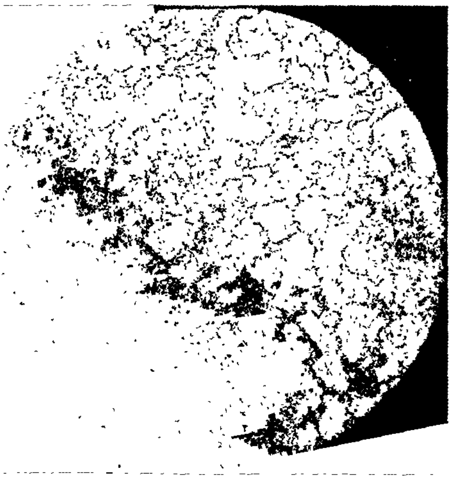


Fig. 7.—Photomicrograph of rat's lung after 60 days infection with H 37. Several cell aggregates constituting the pulmonary tubercle in the rat are shown.
Hematoxylin and eosin, 4 mm. objective, 5 eye piece.

inoculation, the testing dose being 0.5 c.c. old tuberculin diluted with an equal volume of salt solution and injected intraperitoneally. Noninfected controls received like doses, and in some instances double the dose was given, without any effect, save for some slight symptoms, due to the irritant action of the glycerin which lasted for a few minutes. Many of the infected animals, on the other hand, became quite ill within a few hours of the injection, and many died within twenty-four hours. After the lapse of twenty-four to forty-eight hours the surviving animals usually appeared to have recovered completely.

The results of these experiments are given in Table I, which shows that the rat infected with the tubercle bacillus develops tuberculin hypersensi-

tiveness, and this is particularly apparent in animals on diets inadequate in vitamin *A*. With the exception of this deficiency, the diets were adequate in other respects. The protein in the diet of lots 56 to 61 (Table I) consisted of 40 per cent rolled oats supplemented with 10 per cent purified casein; 18 per cent purified muscle protein was the sole protein source in the diet of lots 38 and 39; and in the diet of the remaining lots 18 per cent purified casein furnished the protein moiety. Five per cent dried baker's yeast furnished the vitamin *B* requirement of the last two groups, while in the first group the animals derived this accessory from the oat kernel.

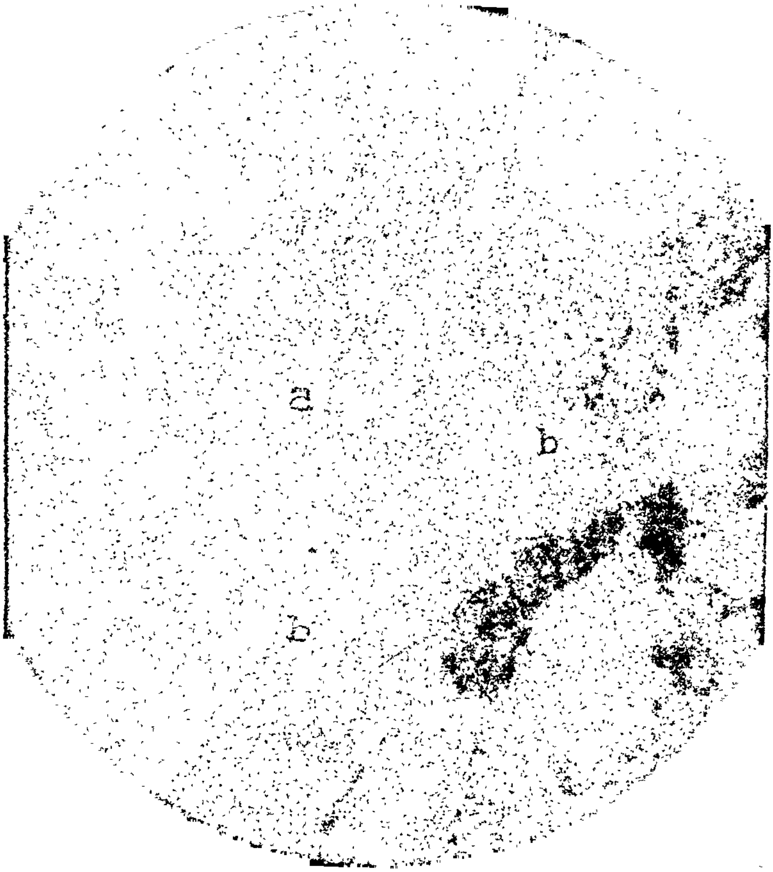


Fig. 8.—Higher [illegible] one of the cell aggregates shown in Fig. 7. The cell aggregate consists of [illegible] and multinucleated cells (*b*). Lymphocytic infiltration is seen throughou[illegible] but more especially around the multinucleated cells. Hematoxylin and eosin, 4 mm. objective, 5 eye piece.

It is evident from the data given in the accompanying table that the adequately nourished tuberculous rat is relatively insusceptible to tuberculin shock. The precise nature of tuberculin shock is as yet unknown, but it may be of interest to point out that the rat is relatively insusceptible to other forms of shock, such as anaphylactic shock,[15] shock produced by diphtheria toxin,[16] and traumatic shock.[17] The recent work of Voegtlin and Dyer[17] on the enormous resistance of the rat to histamine and to the active principle of the infundibular lobe of the pituitary gland as well as to traumatic shock

TABLE I

EFFECT OF OLD TUBERCULIN ON TUBERCULOUS RATS MAINTAINED ON DIETS VARIOUSLY SUPPLIED WITH VITAMIN A. HALF C.C. O. T. DILUTED WITH AN EQUAL VOLUME OF SALT SOLUTION, INJECTED INTRAPERITONEALLY

VITAMIN A (BUTTER FAT) PER CENT	LOT	NUMBER OF ANIMALS	DAYS SINCE INOCULATION	WEIGHT IN GRAMS	NUMBER DYING	MORTALITY PER CENT
Infected with B. tuberculosis, H 37, 5 mg. intraperitoneally						
0	38	4	97		3	
	65	6	50		2	
	66	4	65	85–140	2	50
1	57	5	165		3	
	68	6	50	90–135	6	82
2	39	5	97		4	
	59	6	155		5	
	70	6	50	67–155	3	70
3	72	6	50	95–165	2	33
5	61	4	155	105–160	1	25
Noninfected controls						
0	64	5	—	97–125	0	0
1	56, 67	7	—	100–170	0	0
2	58, 69*	11	—	93–190	0	0
3	71	6	—	93–153	0	0
5	60	5	—	115–170	0	0

*Six animals of this group received 1 0 c c. undiluted O.T.i.p.

throws much light on this important problem. These authors conclude from their work that the smooth muscle and the capillaries of this species react to shock-producing toxins in some peculiar manner different from those of other animals. Tuberculin shock may possibly belong to the same category, and the resistance of the rat to this form of shock might be explained on a similar basis. It may be added that from some work that is now in progress on pharmacologic studies of vitamin action, it is becoming quite evident that vitamin *A* deficiency affects in a specific manner the resistance of the white rat to certain poisons, and some evidence has been secured indicating that the vitamin *A* deficient rat shows an increased susceptibility to histamine in much the same manner as to tuberculin shock.

THE INFLUENCE OF VITAMIN DEFICIENCIES ON THE WHITE RAT EXPERIMENTALLY INFECTED WITH B. TUBERCULOSIS

In these experiments it was sought to determine the effect of diets deficient in either the fat soluble vitamin *A* or the water soluble *B* accessory on the course of tubercle infection in the rat. Animals approximately one month of age and weighing about 40 to 50 grams were selected from our breeding colony and separated into groups usually of six each. The animals of a given series were placed on a constant basal ration the food accessory under examination being added in varying and definite amounts to the basal ration. Each of the food mixtures was fed to each of two groups of animals of one sex and as nearly alike in age and weight as possible. After a week or two on the particular diet, the animals of one of the two groups were infected with H 37, as described in the earlier part of the paper, while those of the

other group were kept as controls. The feeding was continued, and, except for weekly weighings of the controls as well as of the infected animals, no further experimental procedures were applied. Histologic sections were made of the tissues of the infected rats that came to necropsy during the course of an experiment or at its termination. It was hoped that some information of value might be gained from a careful study of the tissue reaction and of the growth curve of the infected rat as compared with the noninfected control maintained on diets variously supplied with either vitamin *A* or *B*.

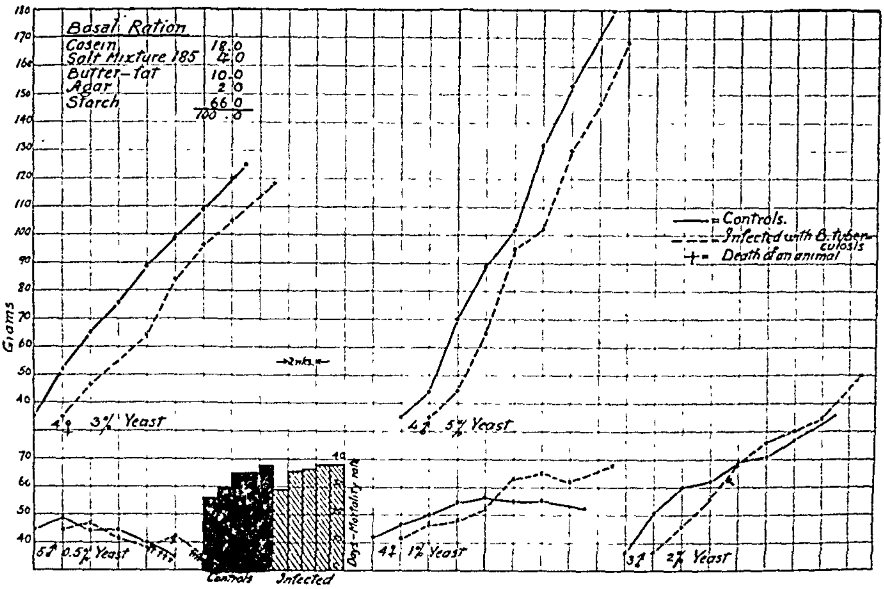


Chart III.—Comparative rate of growth of infected and noninfected controls on varying amounts of water-soluble vitamin *B*, added to a *B* free basal ration. The infected animal in no way differs from the noninfected control as regards its needs for the growth promoting water-soluble food accessory. The mortality rate of the two groups of animals on the *B*, deficient diet (0.5 per cent dried baker's yeast) is the same.

The experiments with the water soluble growth promoting and antineuritic factor were carried out according to the plan outlined above, using a basal ration of the following composition:

Casein (purified by the method of McCollum[18])	18.0
Salt mixture 185[19]	4.0
Clarified butter fat	10.0
Agar	2.0
Starch	66.0
	100.0

The water soluble food accessory was added in the form of dried baker's yeast in amounts ranging from 0.5 per cent to 5 per cent, replacing equivalent amounts of starch in the basal ration. The results, which are shown in Chart III, indicate that the rate of growth of the infected rat is not affected differently from that of the noninfected animal by vitamin *B* deficiency. The mortality rate was practically the same in the two groups which were fed a mixture containing a percentage of yeast below the minimal requirement for maintenance. The histologic findings of the infected animals of the several groups bore a relation to the length of infection, but there was nothing to

suggest an altered tissue reaction to the tubercle bacillus that could be ascribed to the dietary deficiency.

The observations made with the antixerophthalmic and growth promoting food accessory yielded results which were somewhat more encouraging. The earliest experiments conducted on this phase of the nutrition problem were made with a basal ration, slightly modified from that of McCollum, Simmonds, Becker and Shipley,[20] containing the following:

Rolled oats	40.0
Casein (purified)	10.0
Sodium chloride	1.0
Calcium carbonate	1.5
Olive oil	10.0
Starch	37.5
	100.0

Vitamin *A* was furnished in the form of clarified butter fat in amounts ranging up to 10 per cent, replacing equivalent amounts of olive oil. Inspection of Chart IV, summarizing the result of this experiment, will reveal that the infected animals fell distinctly behind the noninfected controls in the rate of growth on the lower allowances of butter fat, but made nearly as good a growth as the controls on a diet containing 10 per cent butter fat. At necropsy the usual degree of tuberculous involvement was found in the infected animals of this series, and histologic examination of the tissues failed to reveal anything different from the cellular reaction to the tubercle bacillus in the adequately nourished infected rat. These findings, which were reported in an earlier preliminary communication,[1b] were interpreted to mean that the rat infected with the tubercle bacillus requires more than the normal quota of vitamin *A* for its growth. Further observations on the relation of vitamin *A* to tubercle infection make the correctness of this interpretation somewhat doubtful, since it has not been possible to duplicate the results on a basal ration containing 18 per cent casein (for the composition of this see Chart I), supplemented with graded amounts of butter fat or cod-liver oil.

Examination of the weight curves of Chart IV makes it evident that the growth curves of the control animals are decidedly below normal, even on mixtures containing considerable butter fat. This becomes particularly clear upon examination of the individual weight curves of the animals of a group, when considerable variation in the rate of growth is noted. Growth of the rat is far more uniform and proceeds at a greater rate on a ration containing 18 per cent casein as its protein (see Chart I). The inferiority of oat-casein mixtures to an 18 per cent casein mixture in the nutrition of the rat was indeed pointed out several years ago by McCollum, Simmonds and Pitz.[21] At first sight it appeared that the phosphorus of the oat-casein mixture of Chart IV might have been somewhat low. A series of experiments was therefore carried out with the basal ration as shown in Chart IV with the addition of 1.5 per cent of monobasic sodium phosphate. The experiment was conducted for a period of eighteen weeks, at which time the average weight gains of the several groups were as follows:

BUTTER FAT P.C.	NONINFECTED Grams	INFECTED Grams
1	45-108	43-91
2	30-116	30-77
5	25-130	25-96

The animals on this diet grew even more slowly than those of Chart IV. This was probably due to their younger age and smaller size when placed on the diet.

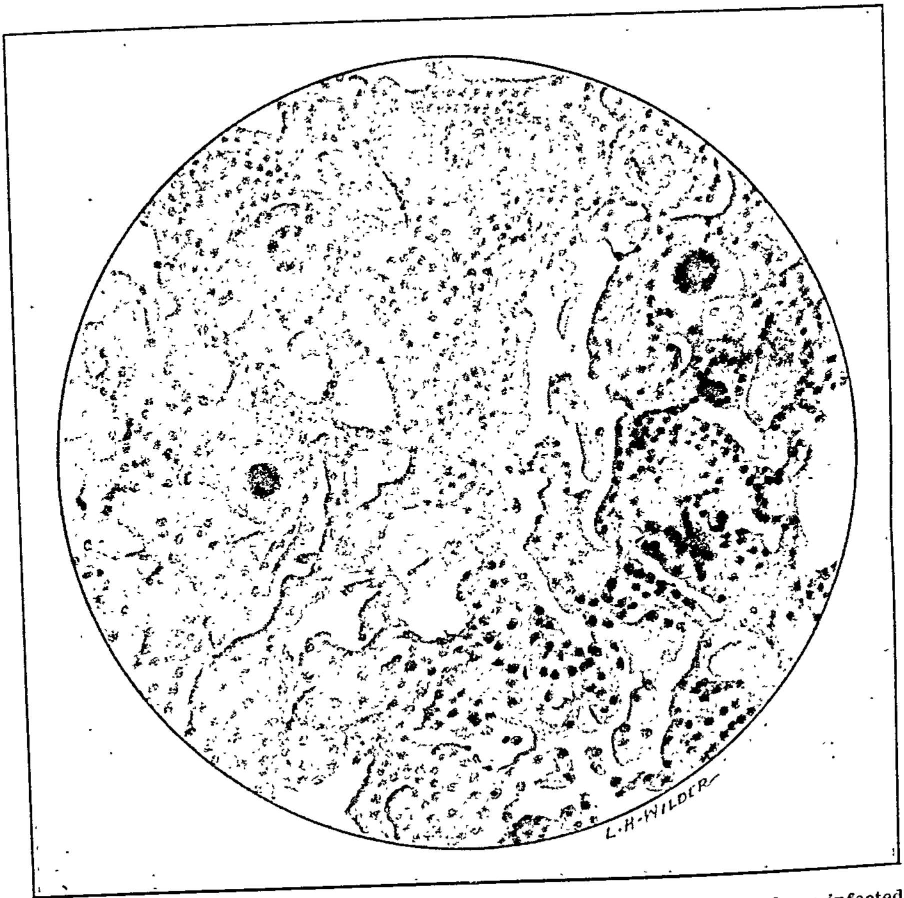


Fig. 9.—Drawing of 4 mm. objective and 10 eye piece magnification of rat's lung infected with B. tuberculosis 6 months. Epithelioid, multinucleated, and several typical giant cells are shown.

On further consideration it seemed probable that the deficiency of the oat-casein mixture might be due to inadequate supply of the water soluble vitamin. A third series of experiments were carried out with the same basal ration as shown in Chart IV with the addition of 2 per cent dried brewer's yeast. Considerable improvement in growth was effected by this addition. Whether this is due to the yeast protein or the water soluble vitamin cannot be stated at present. Here again as in the preceding experiments, the vitamin *A* deficiency was far more pronounced in the infected rat than in the noninfected control. The infected rats on the basal ration without butter fat (Group 111, Chart V) showed an extreme degree of malnutrition, character-

ized by loss of hair, marked emaciation, eczematous condition of the skin of the head and neck, besides the usual eye lesions. The animals of this group deteriorated rapidly, and most of them died within three to four months of the infection, while the controls, though showing some individual variation in the rate of growth, nevertheless presented a relatively fair state of nutrition. Fig. 14 shows the marked contrast in appearance of one of the infected animals (b) in group 111, and (a) a representative of the noninfected group

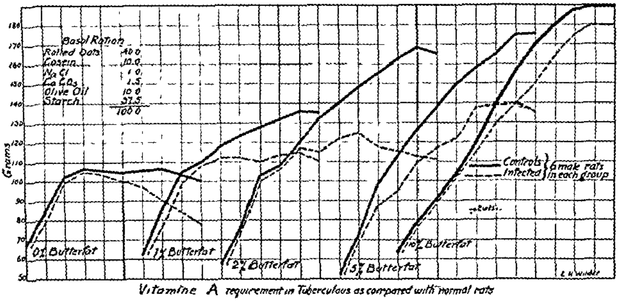


Chart IV.—Shows comparative rate of growth of infected and noninfected controls on varying amounts of vitamin *A* added to a basal ration nearly free from this vitamin. On a low allowance of vitamin *A* the growth rate of the infected rat is lower than that of the noninfected animal. With an increased allowance of butter fat the nutrition of the infected rat, as shown by the weight curve, is improved during its growth period and tends to approach that of the noninfected control.

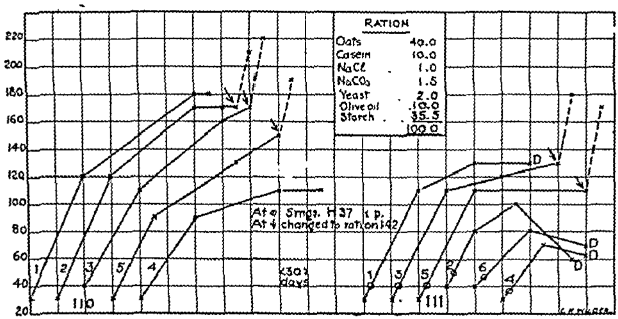


Chart V.—Course of tuberculous infection in rats maintained on a diet as of Chart II, but low in vitamin *A*. The infected animals (group 111) failed to grow anything like the controls, developed early cachexia and most of them died within three to six months after infection. At the point indicated by the arrow three of the controls and the two survivors of the infected group were put on a ration (similar to that of Chart I) adequate in all respects. Prompt resumption of growth occurred.

110, both of Chart V, maintained under identical dietary conditions. Rat C is included in the photograph to show the splendid state of nutrition of an infected animal on a food mixture including 10 per cent butter fat. The animals had been on the respective diets four months when the photograph was taken.

The results of the three series of experiments with the oat-casein food mixtures without butter fat or with the addition of a minimal amount thereof have consistently shown a much more marked degree of deterioration and decline in the case of the tubercle infected rat than in the noninfected control. The results furthermore indicate that with the inclusion of liberal amounts of butter fat in the oat-casein ration the differences in growth and in the general appearance of the infected and the noninfected animals tend to disappear during the growth period of the animal. It is not possible to state with certainty to what the early deterioration of the infected rat on the

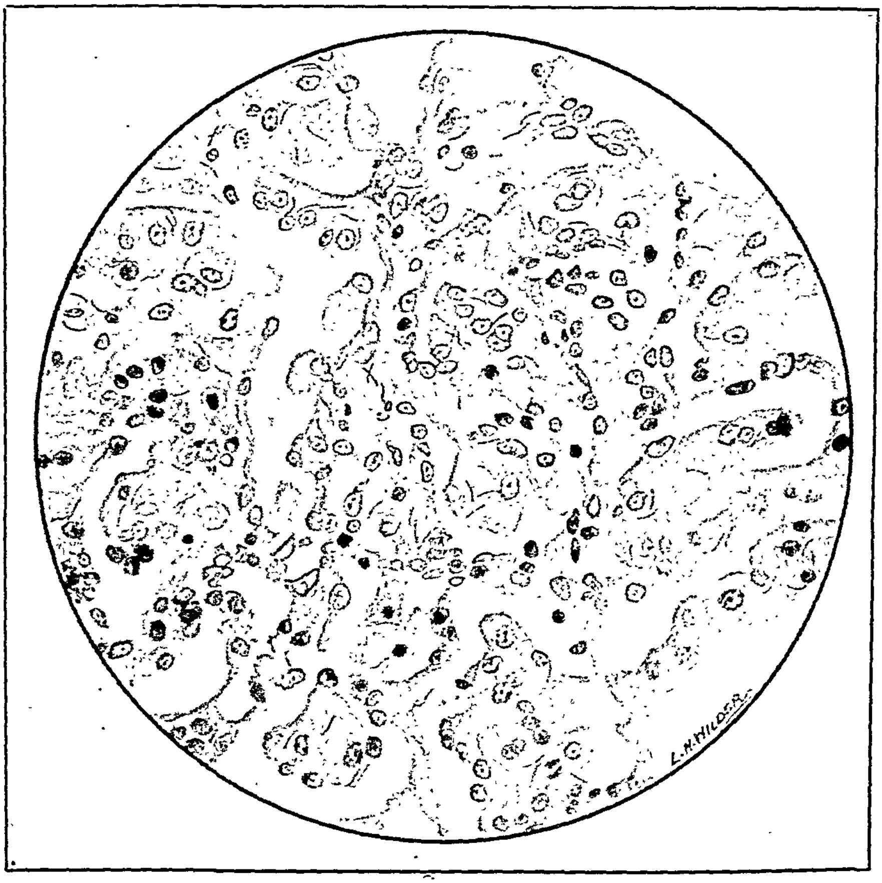


Fig. 10.—Section of lung as of preceding figure, stained with hematoxylin, carbolfuchsin and orange *G*. Shows many tubercle bacilli within the epithelioid and multinucleated cells. 1.9 mm. objective, oil immersion, 5 eye piece.

vitamin *A*-free oat-casein food mixture is due. While the symptoms are suggestive of an extreme degree of malnutrition, they are not unlike the cachexia of the terminal stages of tubercle infection in the adequately nourished rat, developing many months after inoculation. The observed facts appear to be susceptible of two interpretations. Either the tubercle bacillus multiplying in the tissues of the rat renders the latter more susceptible to vitamin *A* deficiency under certain dietary conditions; or vitamin *A* deficiency, in conjunction with a diet of low biologic value, decreases the resistance of the rat to tubercle infection to such a degree as to cause the appearance of grave

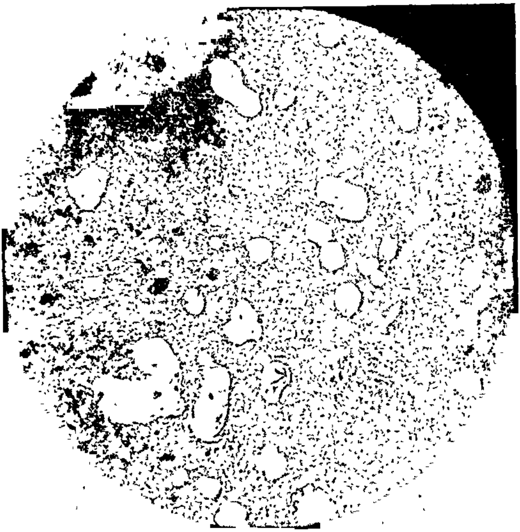
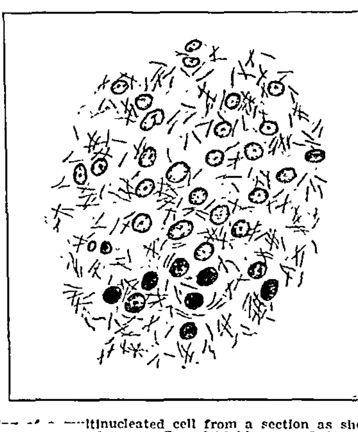


Fig. 11.—Photomicrograph of the lung of a rat that died from tuberculosis nine months after infection. The entire lung tissue is replaced with syncytial masses of multinucleated cells, nearly completely obliterating the air spaces.
Hematoxylin and eosin, 16 mm. objective, 5 eye piece.



[illegible] ltinucleated cell from a section as shown in Fig 11, stained with [illegible] nd orange G, and highly magnified. Note the enormous numbers [illegible] cell, which has not apparently undergone any degenerative chan[illegible] bjective, 5 eye piece.

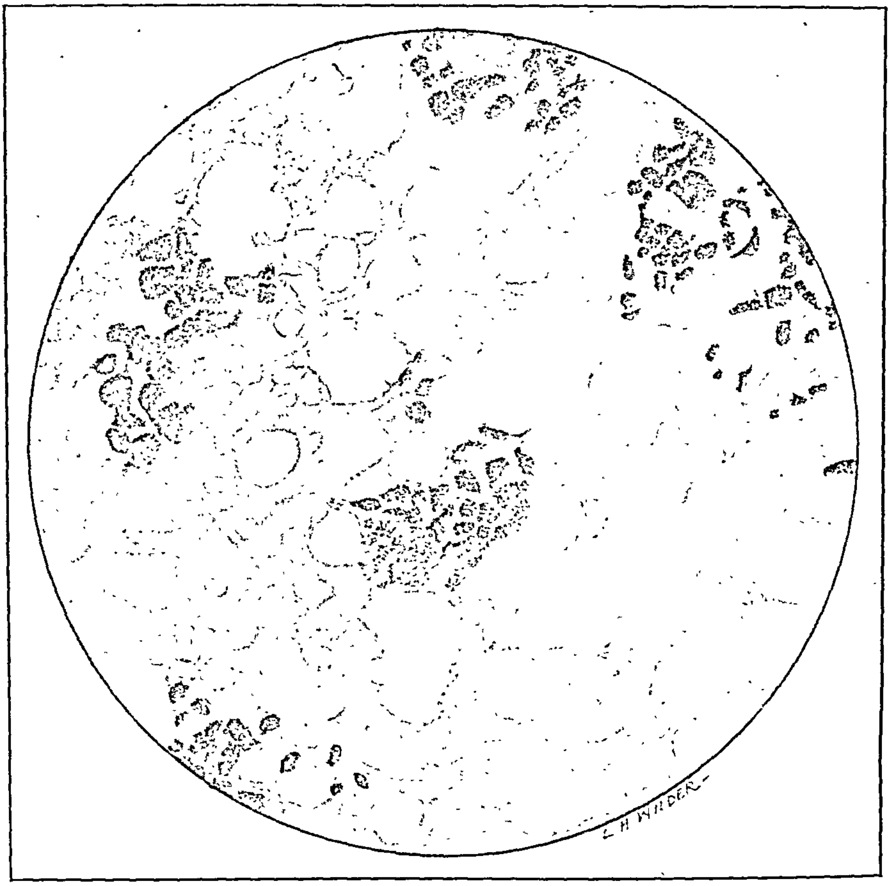


Fig. 13.—Drawing of a fresh frozen section of rat's lung infected with B. tuberculosis 5 months. The section which was stained with sudan III, shows the lipoid changes in the cell aggregate constituting the pulmonary tubercle in the rat. 16 mm. objective, 10 eye piece.

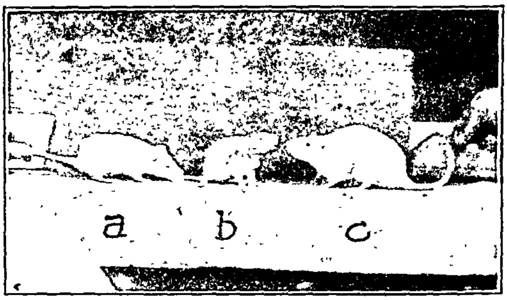


Fig. 14.—Photo of a representative rat of control group 110 (*a*), and one of the infected group 111 (*b*), both of Chart V. These rats have been for four months on a diet low in vitamin A. Rat (*c*) represents one of a group of infected animals having been the same length of time on a diet adequate in all respects.

symptoms in about one-third the time as compared with that required for their appearance in the adequately nourished animal. It should be noted that the increased susceptibility just referred to whether it be an increased susceptibility to vitamin *A* deficiency, or a lowered resistance to tubercle infection in the *A*-deficient animal, has been successfully demonstrated only on the oat-casein mixture, a diet of relatively low biologic value. The relation of vitamin *A* deficiency to tubercle infection is not clearly apparent on a ration so constituted as to be adequate in all respects except the vitamin *A* factor. Thus, on the 18 per cent casein basal ration variously supplied with vitamin *A* in the form of butter fat or cod-liver oil, early nutritional failure in our experience is not apparent until practically all vitamin *A* is withheld, when the infected rat apparently deteriorates more rapidly and succumbs sooner than the noninfected one.

The precise rôle of other nutritional deficiencies in the relation of vitamin *A* deficiency to tubercle infection cannot be indicated at present. A more intimate knowledge of the nature of the oat-casein deficiency, or more properly the oat deficiency, would undoubtedly help in this matter. The work of McCollum, Simmonds and Pitz[21] on the dietary deficiencies of the oat kernel in the nutrition of the rat is helpful in understanding the observed differences in the behavior of the infected rat on the oat-casein basal ration on the one hand and on the 18 per cent casein ration on the other.

SUMMARY AND CONCLUSIONS

Experimental tuberculous infection in the albino rat is described.

It is shown that the albino rat, experimentally infected with B. tuberculosis, can be rendered susceptible to tuberculin shock by withholding fat soluble vitamin *A* from its diet.

Experiments are described indicating that the tubercle infected rat deteriorates more rapidly than the noninfected control when maintained on a diet of low biologic value and low in fat soluble *A*. Liberal allowance of fat soluble *A* appears to afford protection against the early deterioration. It is not certain whether this indicates a lowered resistance in the albino rat to tubercle infection or an increased demand for vitamin *A*. The increased susceptibility to tuberculin shock of the infected vitamin *A* deficient rat appears to offer evidence in favor of the former view.

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CARCINOMA OF THE STOMACH WITH HIGH BLOOD EOSINOPHILIA*

By Emil Weiss, M.D., Chicago, Ill.

EOSINOPHILIA, or the increase of eosinophilic polymorphonuclear leucocytes in the blood is observed under various circumstances, normal and pathologic. As a rather rare occurrence eosinophilia has been observed in connection with malignant tumors. Several authors have found a moderate eosinophilia (5 to 15 per cent) in cases of malignant tumors, but eosinophilia above 15 per cent has been found only in a few cases. Reinbach was the first to report a carcinoma of the neck with a high eosinophilia in the blood. The white blood count was 120,000 with 48.28 per cent eosinophiles. Three weeks later the white blood count went down to 52,000 with eosinophiles varying between 37.70 per cent and 46.15 per cent. According to Reinbach there are three factors which probably cause eosinophilia: (1) decrease in function of the blood-forming apparatus, (2) positive chemotaxis of toxins produced by the tumor cells, (3) disturbances in forming of white blood cells with a larger number of cells in circulation. Vosswinkel and Baradulin observed in cases where the tumors were removed a definite decrease of the acidophile cells. Kappis describes a case of carcinoma of the right lung with eosinophilia 33.6 per cent to 35.5 per cent. The histologic examination of the tumor tissue in this case showed strong infiltration of eosinophiles in necrotic areas. Many eosinophiles and eosinophile myelocytes were found in the tumor free bone marrow. According to Kappis, the necrotic tumor tissue exerts a chemotactic influence and causes a specific hyperproduction of eosinophiles and a secondary infiltration in the necrotic tumor tissue. Donati reports in his paper, among other cases, a carcinoma of the breast with 20 per cent eosinophiles; Dunger reports a carcinoma of the neck with 21,290 eosinophiles (38,330 leucocytes), with 21,400 eosinophiles (27,600 leucocytes), eight days later. Strisower described a carcinoma of the cervix with eosinophiles varying from 4.4 per cent to 45.37 per cent; Csaki, a malignant tumor of the colon with 30 per cent eosinophiles. Zappert, Oshima, Collins and Kaplan observed cases of sarcoma with high eosinophilia. (17.76 per cent to 31 per cent.)

The case which the writer wishes to report in this paper may be of considerable interest because of the fact that (1) it is the first reported case of carcinoma primary in the stomach with high eosinophilia; (2) there was opportunity to follow the case for a reasonably long time; (3) experimental work was done in connection with this case.

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A. C., a female of forty years, came into the hospital April 7, 1925. Her menses began at the age of fifteen and were always regular. She had six children, three living and three who died very shortly after birth of childhood diseases. Since the birth of the last child, she complains about pain in the epigastrium. Otherwise she has never been sick. She lost twenty pounds in the past two years, five pounds in the last three months. The present complaints are chiefly localized in epigastrium. The pain in this region grows much worse when she eats. She vomits frequently, especially after large meals; even milk is vomited and curdled. The vomiting occurs usually half an hour following meals, at other times two hours after. Patient vomited blood twice in the past year. She has a good appetite; is very often constipated; is very anemic, and shows no disturbances of cardiorespiratory or of genitourinary apparatus.

The physical examination revealed apparent chronic illness, pallor and some emaciation. Eyes react to light, left eye does not respond to light as the right one. Tongue, teeth, mouth, neck, heart and lungs normal. Abdomen very soft and flaccid, with four distinct masses, palpable. (1) The largest and hardest mass was located about one and one-half inches below the costal margin, slightly to the left of the midline, in the epigastric region. (2) The second palpable mass was also hard and movable, located in the right hypochondrium; it was free from the liver, probably attached to the pylorus or gall bladder. (3) The third mass was hard and located in the left hypochondrium. (4) The fourth mass was in the umbilical region about one inch below the navel in the midline.

The x-ray report showed fluoroscopically a marked filling defect involving the entire pyloric region; it was spiculated and obstructive in its outline, typical of a neoplasm. Radiographic films conform to the above findings; some, six hour stomach stasis. Conclusion: characteristic evidence of advanced carcinoma.

Several laboratory tests were made. Blood examinations were made as shown in Table I.

TABLE I

DATE	RED BLOOD CELLS	WHITE BLOOD CELLS	HEMOGLOBIN	DIFFERENTIAL COUNT: LARGE AND SMALL LYMPHOCYTES	MONONUCLEAR AND TRANSITIONAL	POLYMORPHS: NEUTROPHILE	EOSINOPHILE	BASOPHILE	MYELOCYTES AND MYELOBLASTS
4–10 1925	4,240.000	16.300	70%	6.8	1.5	67.5	24.2	–	–
4–23	4,050.000	7.300	60	7.5	2.7	85.4	4.1	–	–
5–16	4,120.000	6.900	60	19.8	6.2	48.3	25.7	0.3	–
5–23	4,100.000	6.100	55	18.6	5.2	44.4	31.8	–	–

In the first differential count were found only a few megalo- and poikilocytes and a few micro- and normoblasts. In the following differential counts the number of immature red cells became gradually larger. Other laboratory tests were made as follows: Wassermann test was negative; 4 feces examinations for parasits were negative; 9 urine examinations showed a slight trace of albumin in a few instances, as a result of the increased number of pus cells.

Ten days after entrance into the hospital, the patient was operated upon. The lower half of the stomach was resected and the upper part, directly connected with the duodenum. The gross examination revealed a large tumorous mass occupying the lower half of the stomach, located chiefly on the posterior wall of the stomach and extending from the lesser curvature to the major and obstructing the pylorus. The surface of the tumor mass was soft and necrotic, the deeper parts were more resistant. No metastases could be detected in the abdominal organs. The microscopic examination of the tumor showed a typical adenocarcinoma, penetrating the mucosa, muscularis and

serosa. The connective and muscle tissue surrounding the carcinoma was to a great extent necrotic with no eosinophilic infiltration in this necrotic tissue.

EXPERIMENTS

The tumor mass was carefully cut out, ground and extracted with saline, which was added in the proportion 1:5. The flask was shaken and then put in the ice box for several days. The supernatant fluid was then injected intraperitoneally, intravenously, and intracardially in guinea pigs, rabbits and dogs, respectively. Complete blood counts were made on the animals previous to, one and three hours, and one, two and four days after the injections. The white blood cells showed a rise of two to three times as high as previous to injection after intracardial or intravenous injections. The leucocytosis disappeared after a few hours. Intraperitoneal injections showed a rise of white blood cells after two to three hours, usually disappearing completely within twelve hours. The differential count did not reveal in any of these injections an increase of eosinophiles; only the so-called pseudoeosinophiles in rabbits and guinea pigs showed a marked rise, while the dogs showed only a neutrophile leucocytosis.

In order to determine whether or not the blood of the patient contains toxic substances, derived from the tumor to cause eosinophilia, 2 c.c. of the patient's blood was defibrinated with an equal amount of a two per cent sodium citrate solution and injected in the ear vein of a dog. The white blood cells showed a 100 per cent increase three hours after injection. The differential counts revealed a high neutrophile leucocytosis. Not the slightest rise of eosinophiles could be observed. Forty-eight hours after the injection the white blood cells showed normal conditions.

To determine whether or not the eosinophiles possessed any phagocytic properties the defibrinated blood of the patient was washed three times with saline; after the supernatant fluid was removed, only the upper layers of the blood, which contained most of the leucocytes, were used for the study of phagocytosis. One mixture was prepared by mixing equal amounts of blood, typhoid bacilli and saline. The other mixture contained equal amounts of blood, typhoid bacilli and typhoid immune serum. Both mixtures were placed for half an hour in a water-bath at 37° C. After incubation, smears were prepared from both mixtures, stained with Wright's stain, and covered with Löffler's methylene blue for a few seconds. Eosin-hematoxylin and methylene blue proved to be as useful. No smear of either series showed eosinophile leucocytosis, while the neutrophiles were crowded with bacteria.

DISCUSSION AND SUMMARY

The writer presents a case of carcinoma with high eosinophilia in the blood. In the past only six cases of carcinoma with such high eosinophilia have been reported in the literature. In none of the previous cases was the tumor located in the stomach. In one of these cases, an eosinophilia was found in the surrounding necrotic tissue and a direct connection was assumed between the necrotic tumor tissue (toxins) and the eosinophile infiltration.

No experimental work was done to substantiate the above theoretic assumptions. In the case under discussion no eosinophilia was found in the tumor or surrounding tissue. Similar to the observations of Vosswinkel and Baradulin a decrease of eosinophiles after the removal of the tumor was observed. These two authors had no opportunity to observe their cases during a longer period following the operation. In our case a few weeks after the operation the eosinophiles showed again a rise exceeding the eosinophilia previous to the operation; whether this postoperative eosinophilia was caused through metastatic growth of the tumor, could not be determined. Neither the tumor extract, nor the blood of the patient could produce eosinophilia in animals (guinea pigs, rabbits and dogs). Our experimental work could not confirm the theoretic assumptions of Kappis, who thinks that the eosinophiles in the blood and in the tissues were caused by the toxins, derived from necrotic tumor tissue. Not the slightest phagocytic properties of the eosinophiles, even in the presence of immune serum, could be detected.

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A PLEA FOR A STANDARDIZED METHOD OF ESTIMATING AND REPORTING HEMOGLOBIN VALUES*

By Janvier W. Lindsay, M.D., E. Clarence Rice, M.D., and Maurice A. Selinger, M.D., Washington, D. C.

THE average routine blood examination usually includes an estimation of hemoglobin, and in the diagnosis of various types of anemias the clinician is in a great measure dependent upon a knowledge of the amount of this substance for a correct diagnosis.

Unfortunately for the general practitioner, a variety of instruments using different standards are on the market and are being used. Kern,[1] from the viewpoint of the internist, has shown how the variety of standards and the estimating of hemoglobin in per cent has served to make this examination of the least value in the routine blood examination. Many are not aware of the fact that each type of hemoglobinometer usually has a different value for its 100 per cent of hemoglobin. Chart I gives a curve setting forth the variety of standards of different observers, each representing 100 per cent.

If three different hematologists, each using a different type of hemoglobinometer, assuming each to be accurate, were to estimate the hemoglobin percentage of a patient, it would be difficult to convince the average attending physician that the following results were correct by the method used and that each represented the same amount of hemoglobin in grams per one hundred cubic centimeters:

Sahli-Leitz	100% = 15.00 gm. per 100 c.c.
Dare	109% = 15.00 gm. per 100 c.c.
Newcomer	88% = 15.00 gm. per 100 c.c.

A situation such as this frequently does arise when a patient is transferred from one hospital to another where different hemoglobinometers are used, with the result that complaint is often made that the hemoglobin estimation at one or both laboratories was incorrect. This misunderstanding could be overcome if the result was given in grams per 100 c.c. as well as per cent.

Many physicians have been in the habit of thinking of the amount of hemoglobin in an abstract way. One hundred per cent to them is equivalent to 5,000,000 erythrocytes per cubic millimeter, but they would ordinarily be at a loss to explain just what 100 per cent represents.

Inasmuch as it is well known that the hemoglobin content of the blood varies with the age, sex and time of day, it seems logical to allow considerable variation for the normal limits. Various observers (Dare, Haldane, Haden,

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Sahli, Williamson) have taken their normals from approximately 14.0 to 17.0 gm. per 100 c.c. and, in establishing an average, 15.6 gm. per 100 c.c. is generally accepted. The limits of 14.0 to 17.0 gm. per 100 c.c. is therefore suggested to be reported as the limits of normal when giving the hemoglobin content of the blood, the amount in grams per 100 c.c. as well as the percentage being reported as a routine. In the chemical examination of the blood a considerable leeway is allowed in the matter of normals, for example, sugar 80 to 120 milligrams per 100 c.c. of blood, and it is reasonable to assume similar variations for normal blood with reference to the hemoglobin content should be allowed.

The members of this Society would greatly clarify the general misunderstanding among clinicians if they would make it a practice to report hemoglobin values in the manner described.

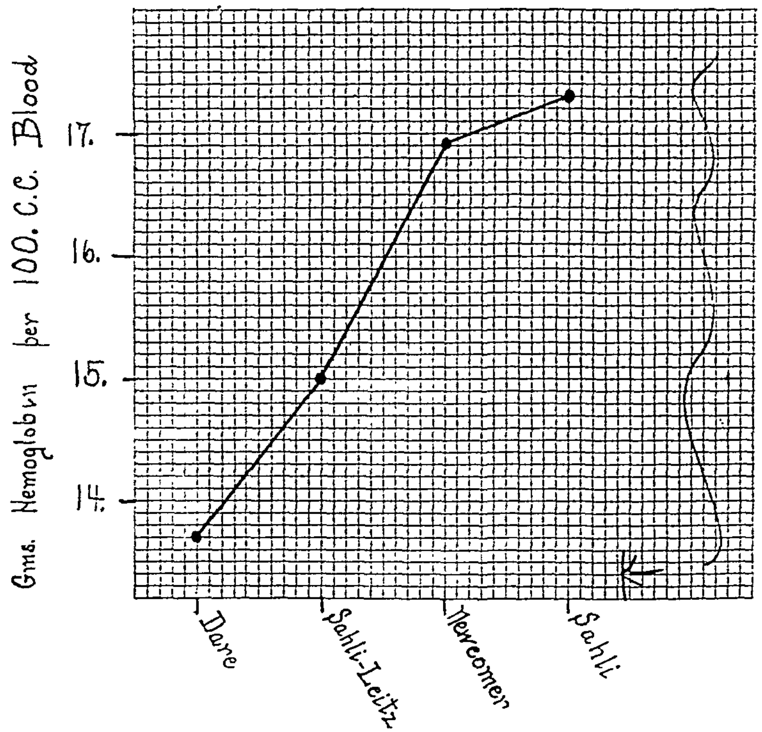

Chart I.—Variation in value of 100 per cent for various types of hemoglobinometer.

As a result of the lack of proper standardization of instruments by the manufacturers or the inability to secure standardized replacements for certain other types, it is often impossible to give accurate results as determined directly without necessitating the use of a table of corrections for each instrument found to give inaccurate readings.

Comparison of the results obtained with the Sahli,[2] Dare,[3] and Newcomer[4, 5] hemoglobinometers with those obtained by Van Slyke's[6] oxygen capacity method and Wong's[7] colorimetric method was made (Table I) and showed such marked variations from the normal with the first two instruments as to make them useless for clinical work without restandardization.

TABLE I

COMPARISON OF ACCURACY OF VARIOUS TYPES OF HEMOGLOBINOMETERS

Instruments used were secured from various physicians' clinical laboratories except "factory Dare," which was obtained direct from the manufacturer

SPECIMEN	SAHLI	DARE A	DARE B	DARE FACTORY	NEWCOMER	WONG	VAN SLYKE'S OXYGEN CAPACITY
1	18.7 (125%)	15.6 (114%)	14.4 (105%)	–	18.3 (108%)	18.8	18.78
2	18.9 (126%)	10.3 (75%)	10.4 (79%)	–	15.2 (90%)	15.79	15.89
3	15.0 (100%)	8.7 (63%)	9.2 (67%)	8.1 (59%)	9.1 (54%)	12.48	12.21
4	4.2 (28%)	2.0 (15%)	–	1.8 (13%)	2.9 (17%)	3.11	–
5	20.2 (135%)	10.7 (78%)	–	–	15.1 (90%)	14.35	–
6	15.7 (105%)	6.8 (50%)	–	–	9.1 (54%)	8.13	9.06
7	15.7 (105%)	7.0 (51%)	–	–	9.3 (55%)	7.89	7.88
Average Error	36.9%	23.7%	27.3%	38.0%	2.1%		

TABLE II

HEMOGLOBIN

WONG'S METHOD	VAN SLYKE'S OXYGEN CAPACITY METHOD	DIFFERENCE
18.80 gm. per 100 c.c.	18.78 gm. per 100 c.c.	+0.02
15.79 gm. per 100 c.c.	15.89 gm. per 100 c.c.	−0.10
15.87 gm. per 100 c.c.	15.16 gm. per 100 c.c.	+0.71
14.85 gm. per 100 c.c.	14.15 gm. per 100 c.c.	+0.70
12.48 gm. per 100 c.c.	12.21 gm. per 100 c.c.	+0.27
8.13 gm. per 100 c.c.	9.06 gm. per 100 c.c.	−0.93
7.89 gm. per 100 c.c.	7.88 gm. per 100 c.c.	+0.01
		Average +0.097 gm.

The instrument devised by Newcomer, using his glass plate standard, is in our experience the most accurate clinical hemoglobinometer which can safely be placed in the average clinician's hands. The parts of this instrument, so far as we have been able to determine, have been accurately standardized and can be replaced without introducing appreciable clinical error. This cannot be said of the Sahli, although a marked improvement in this instrument has been effected by the introduction of a solid glass standard in the place of the changeable liquid standard (Chart II). We have yet to examine a Dare hemoglobinometer giving sufficiently accurate results without being restandardized, and most physicians would not be apt to undertake this.

The physician who desires to standardize his hemoglobinometer need not possess the skill of a gas analyst, inasmuch as Wong's[7] method for determining the iron and hemoglobin content of the blood is sufficiently accurate as compared with Van Slyke's oxygen capacity method for practical work. This test requires not more than twenty minutes for its performance and it is comparable to Folin and Wu's test for estimating nonprotein nitrogen of the blood in simplicity (Table II).

The method of Cohen and Smith is excellent, but is impracticable for many small laboratories, whereas Newcomer's method is simpler and has been shown to be quite as accurate by Senty.[8] We believe that the Newcomer

hemoglobinometer is to be recommended as the instrument of choice in view of its (1) accuracy, (2) relative simplicity, (3) standardization in manufac-

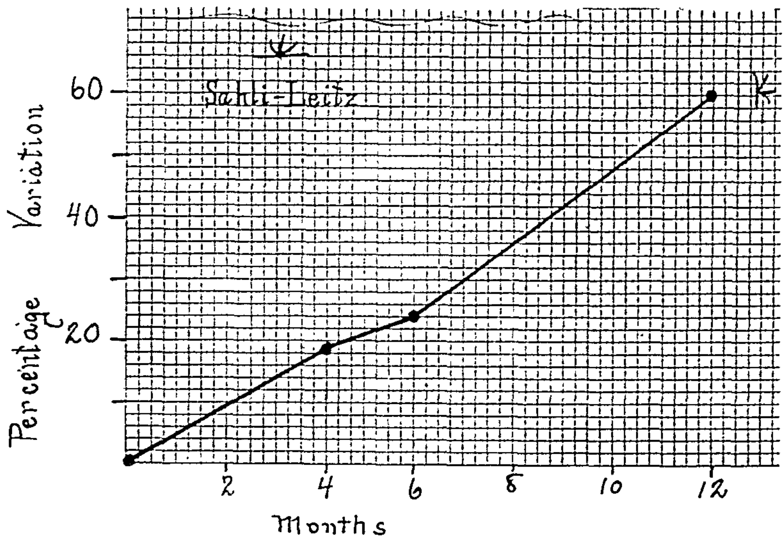

Chart II.—Change due to fading of Sahli standard color tube.

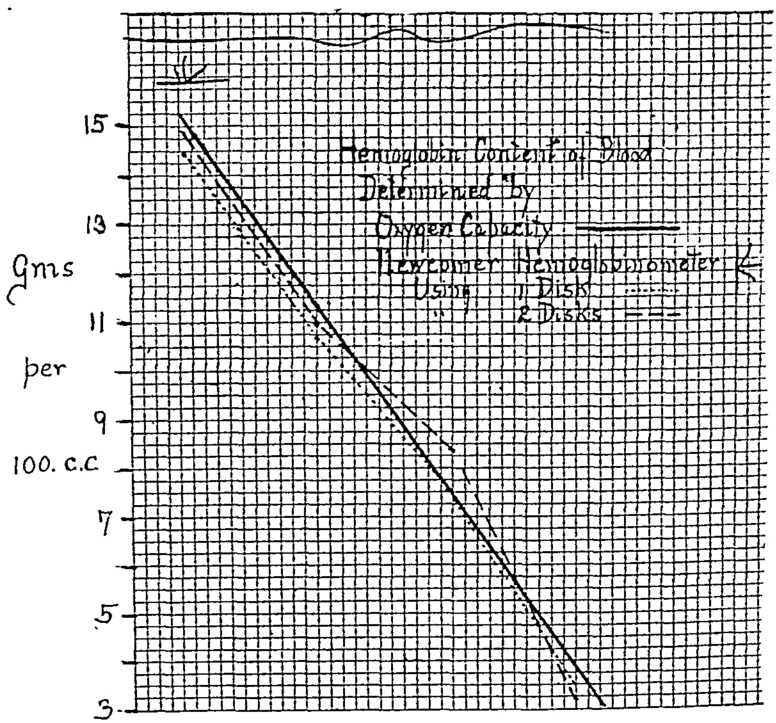

Chart III.—Hemoglobin determinations made with Newcomer hemoglobinometer using one and two color disks, as compared with results by Van Slyke's oxygen capacity method.

ture, (4) the general acceptance of the Duboscq type of colorimeter in colorimetric determinations, and (5) utilization of a color standard with absorption curve similar to that of acid hematin.

One of the objections to the Newcomer disk method has been the difficulty of matching colors, especially when the hemoglobin content of the blood was low. We believe that we have been able to simplify this matter by using two color disks of the same thickness for the standard and double the usual amount of blood (0.02 c.c.). For blood of hemoglobin content less than 6.77 gm. per 100 c.c. (40 per cent Newcomer), 0.02 c.c. of blood is drawn and used with only one of the glass plates as a standard (Chart III).

CONCLUSIONS

1. Few hemoglobinometers on the market are sufficiently accurate for clinical work.

2. Hematologists should make a practice of reporting the hemoglobin content of the blood in grams per 100 c.c. as well as per cent.

3. Fourteen to seventeen gm. per 100 c.c. is suggested as the normal limits of the hemoglobin content of the blood, these figures being used in the same manner as the normals in blood chemistry.

4. Wong's method of determining hemoglobin is sufficiently accurate for standardization of hemoglobinometers in the clinical laboratory.

5. The hemoglobinometer devised by Newcomer using a standardized glass color plate is recommended as the most accurate instrument for clinical work.

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DISCUSSION

Dr. Wm. H. Stoner.—It is common knowledge among laboratorians that there is more poor work done in hemoglobin estimations than in any other test. We checked up a series of approximately twelve Sahli hemoglobinometers that were in use. The Sahli pipettes vary and volumes vary from the calibrations by 15 per cent as a maximum. The dilution tubes vary as high as 10 per cent, and the color comparison tubes vary still more. Sahli is probably more widely used for determining hemoglobin outside the laboratory than any other test. With the advent of Dr. Pons into the Philadelphia Hospital this was looked into, and, through the normal process of evolution, he has arrived at almost the same conclusion as Dr. Rice. This alone is a recommendation for Dr. Rice's method. I think Dr. Rice may be complimented for coming to such a conclusion.

Dr. Carl L. Spohr.—I think we should not overlook the admirable work of Dr. Todd in his textbook on Clinical Pathology in reporting hemoglobin in grams, chiefly with the hope that later on this method might be adopted.

Dr. E. B. Krumbhaar.—When we first started some six months ago to report our hemoglobins in grams per 100 c.c., we were afraid that the clinicians on our staff would not like the method, but we have had no unfavorable comments. We feel that they, like ourselves, will soon become familiar with the reports in grams, so that, for instance, eleven grams of hemoglobin will represent as concrete a picture as, say, four million reds, and that it will not be necessary to transpose into an artificial percentage standard.

Dr. Pons has worked out for us what we feel is the best method of performing the test. It is to have the interne take to the wards a number of tubes of decinormal HCl solution, so that in each case the blood is mixed with the solution right in the ward. We know that a certain amount of time must elapse in order that the color may deepen to maximum capacity. By our method the solution has reached the maximum degree of color in the time it takes to get back to the laboratory, so that when the comparison is made in a standard colorimeter with the Newcomer amber glass plate the margin of error is reduced to a minimum. This test has been found to be foolproof and accurate. I would like to see this Society go on record as approving the method of reporting hemoglobin in grams per 100 c.c. instead of in some more or less arbitrary percentage.

Dr. John A. Kolmer.—I am going to ask the Committee on Laboratory Standardization of which Dr. Sondern is chairman to take these discussions to heart and consider them.

Dr. Frederic E. Sondern.—In going over the broad question of standard methods and in reading the many opinions you have been good enough to send to us, it is evident, as you know, that every laboratory worker has his favorite method which he believes is best for every laboratory procedure. At the same time I quite agree with the thought that committees might be organized to make studies of questions such as this and, while not necessarily establishing a standard for everyone to follow, to present such arguments as would make everyone glad to follow the suggestions. This would probably be justified if the opinion were that of the reporting workers and if their results were expressed in such a way as not to be open to any question. Relative to the matter of estimation of hemoglobin, it is my opinion that this is one of our most unsatisfactory determinations. I know that careful work with different apparatus gives different results and that the same apparatus under the same conditions among different laboratory workers will have readings which vary more than we desire. I wish I could make a recommendation to you which would obviate this.

Dr. Wm. G. Exton.—Dr. Rice certainly deserves to be complimented on his paper. The thought it brings to me is that this Society must take cognizance of the fact that not all of the apparatus and instruments offered by commercial houses are reliable, and that some time or other we should give our attention to finding means of approving those which are reliable. I have had considerable experience with commercial houses and all of them mean well but are apt to be misled. Some years ago we became interested in the various hemoglobin methods and found about the same condition of affairs as Dr. Rice describes. Owing to the fact that our specimens are not always fresh ones we have been depending meanwhile upon iron determinations, which are very simple to carry out.

Dr. C. A. Pons.—For the last year we have been using at the Philadelphia General Hospital, Newcomer's plate as our standard and have been reporting the results in grams. I feel that we are getting more consistent results with this method. If there is any error, I feel that we can put the blame on the person who collected the blood, since we do not have to contend with fading of the standard, precipitation, or inability to match the colors.

We have tried various types of colorimeters, and for our purpose Bausch & Lomb microcolorimeter 3625 has proved very satisfactory. As the normal average for an adult standard we have accepted Haden's standard 15.6 gm. per 100 c.c. of blood.

I believe that this is one side of the story; something has to be done to simplify hemocytometry. I have been very interested in Mr. Trenner's (of the A. H. Thomas Co.) work. He has made a pipette in which the dilutions are doubled and which probably will be very reliable and easy to manipulate. This automatic pipette is similar to the ones in use except that it has no graduations; the blood fills the capillary tube which ends where the bulb begins. Naturally capillary attraction ends here, and this is the correct amount of blood. Suction is necessary to dilute the blood up to the mark at the other end of the pipette. This gives the usual 1:200 dilution.

Dr. Rice (closing).—We have sent several of our color plates to the Bureau of Standards, for a permanent record of their spectral transmission. These will be of value in comparing with similar records made later, or if a new plate is secured, its graph can be compared with that of the original to determine whether it is a duplicate. This work was prompted by Dr. Kolmer's efforts to standardize the Wassermann reaction. I feel that there is often great error in this work and it is up to us to endeavor to eliminate it.

ANISOCYTOSIS AND INCREASED RED BLOOD CELL VOLUME WITH LITTLE OR NO ANEMIA*

By Mortimer Warren, M.D., Portland, Maine

PERSISTENCE of morphologic changes in the red blood cells is characteristic of pernicious anemia at all times during the course of the disease; less marked at the time of remission, these changes are none the less observable in smears. This finding ranks with achlorhydria as indispensable to the diagnosis of pernicious anemia. The determination of the hemoglobin as a presumptive test of the condition of the red blood cells may be misleading, whereas an examination of a properly prepared spread of blood permits immediate decision as to the necessity for further investigation of the patient so far as the blood-forming organs are concerned. With corroborative signs and symptoms, the diagnosis of pernicious anemia is easily established. Lacking this support, the significance of morphologic changes which approach those found in pernicious anemia is uncertain; I am speaking of variation in size of red blood cells with cells distinctly larger than normal, of variation in shape with cells of an oval form, and of cells which give the general impression of hyperchromia, a blood picture which is accompanied by a high volume and color index.

I have selected four cases of this type for presentation by means of microphotographs, using for comparison a case of pernicious anemia in full remission and a case of combined sclerosis with preponderance of cord changes and slight anemia.

METHODS

Hemoglobin by the Sahli method, standardized so that 100 per cent equals 5,000,000 normal red blood cells, occasionally checked by the Bausch and Lomb Newcomer method.

Platelets by the Olaf Thomsen method as described by Gram.[1]

In computing the volume index, the method of Haden[2] was used.

The charts are self-explanatory except that the notation "Poly." means the percentage of polymorphonuclears, and the notation "4" means the percentage of polynuclears having four or more lobes.

Chart I (555/25).—A man of fifty-five years who had been ill for two years at the time of this examination. This is the second remission. The course, history, and signs are typical and require no comment.

Chart II (262/25).—A man of forty-five years, referred by Dr. Henry Swift for lumbar puncture because of absent knee jerks and ataxia. He had some numbness of the legs and, to a lesser extent, of the hands, impairment of position sense, and inability to use his legs without support. The pupils reacted to light. The spinal fluid was clear with one cell per cubic millimeter; a trace of globulin; no curve with gold solution; and a negative Wassermann. These symptoms began two years earlier, following mumps.

*Read before the Fourth Annual Convention of the American Society of Clinical Pathologists at Philadelphia, May 20 to 23, 1925.

CHART III (378/25).—A nurse thirty-four years of age, who has not regained her previous health since having influenza in France in 1917. She looks anemic, tires easily, has considerable flatulence with no abdominal pain, at times legs feel numb with no changes in reflexes, has apparent pallor without icteric tint, tongue is normal.

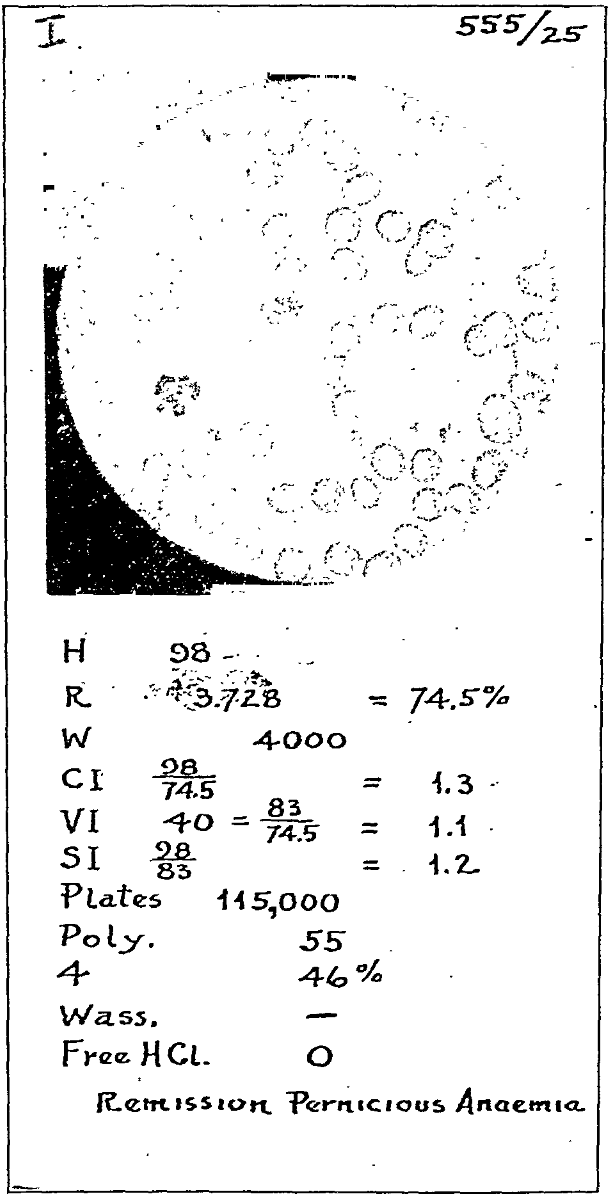


Chart I.

CHART IV (665/25).—A man of thirty whose complaint was of tiring easily. He was told he had malaria in 1917 in North Carolina. Reflexes normal; station normal; tongue negative; joints feel a little stiff. Gonorrhea four years ago. Wassermann strongly positive.

CHART V (753/25).—A woman of twenty-five, patient of Dr. E. W. Gehring, who complained of nausea, weakness, and tiring easily. No physical signs. Basal metabolism (−30). Blood pressure $\frac{110}{75}$. No paresthesia. The characteristic physical and mental features of myxedema were absent.

Chart VI *(795/25).—A man of thirty, a clinic case of cerebrospinal lues with strongly* positive Wassermann in the spinal fluid, a paretic curve, a cell count of twenty, decided trace of globulin. The notation of negative Wassermann on this chart is not representative of the usual state of his blood; under treatment, the Wassermann was reduced, but has since recurred. He has absent knee jerks, stiff pupils, slight unsteadiness, numbness and lightning pains in his legs.

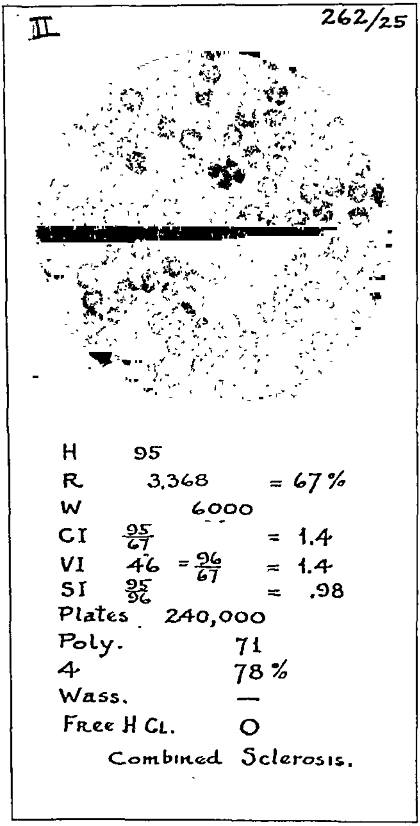


Chart II.

DISCUSSION

In Chart II is represented a type of case with disabling cord lesions and no apparent anemia, to which Woltman[3] calls attention. The blood morphology is sufficiently distinctive to allow of the diagnosis of pernicious anemia.

In fact, the diagnosis rests on the blood picture if all cases of sclerosis are accompanied by achylia gastrica, as found in those reported by Vanderhoof.

The other charts represent dissimilar clinical types, in common having ill-defined disturbance of general health, which in two cases can be attributed

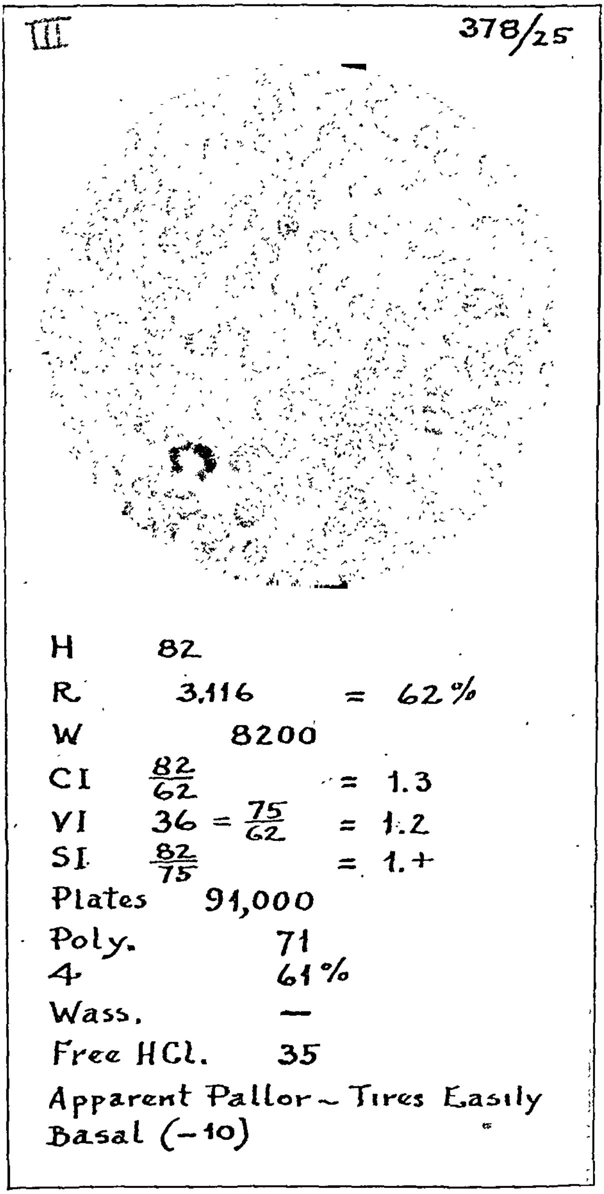


Chart III.

to the constitutional effect of syphilis. These blood findings are suggestive, but can be interpreted with relation to pernicious anemia only in a speculative sense.

According to Hurst and Bell[5] achlorhydria precedes the anemia; may there not be as well a constitutional susceptibility on the part of the marrow

in certain individuals, which manifests itself by changes in the blood in various disturbances of health without the presence of other factors necessary for the development of the completed picture of pernicious anemia?

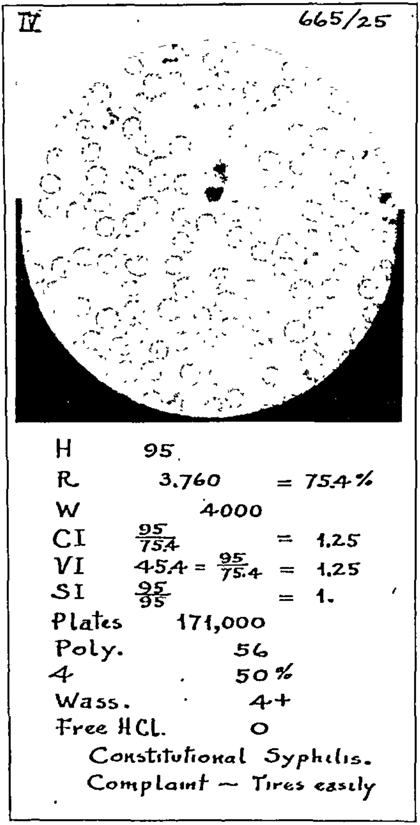
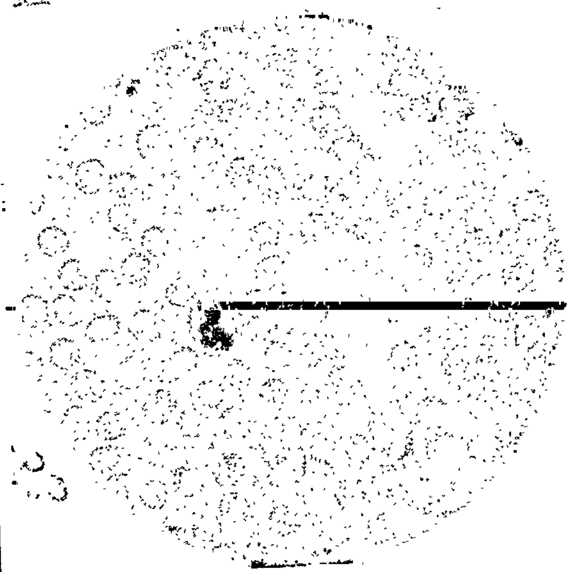


Chart. IV.

SUMMARY

These charts have been shown for the purpose of demonstrating minor changes in blood morphology which simulate those characteristic of pernicious anemia in cases in which the diagnosis of this condition is not confirmable by other data and in which the anemia is minimal.

V 753/25



H 78

R 3.088 = 62%

W 4000

CI $\frac{78}{62}$ = 1.25

VI 33.3 $\frac{69}{62}$ = 1.1

SI $\frac{78}{69}$ = 1.1

Plates 236,000

Poly. 51

4 58%

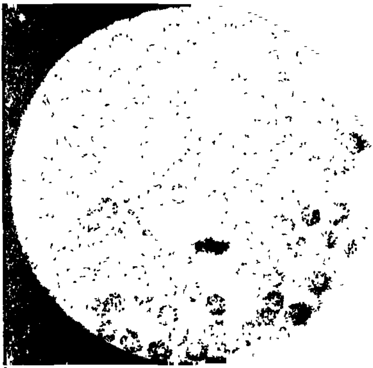
Wass —

Free HCL. 15" = 0 1 hr. = 10

Basal (–30)

Chart V.

VI 795/25



H 80
R 3.728 = 74.5 %
W 4000
CI $\frac{80}{74.5}$ = 1.+
VI 45.7 $\frac{95}{745}$ = 1.27
SI $\frac{80}{95}$ = 0.84
Plates 222,000
Poly. 68
4 40 %
Wass. —
Free HCl. 25
Old Lues ~ Cerebro Spinal

Chart VI.

scopic sections from the tissues obtained at autopsy. Segura and Puccio,[25] in Buenos Aires, reported cultivation of Castellani's spirochetes from the sputum of a patient suffering from an acute pulmonary condition.

1922. Faust[26] reported cases of bronchial spirochetosis in China and stated that a certain proportion of these cases have pulmonary tuberculosis as a primary infection and a secondary spirochetosis. Other cases show simply spirochetosis, usually of the chronic type, although cases of acute infection were observed. Alfaro[27] in Argentina reported four cases of spirochetal infection resembling tuberculosis, one of which had involved the lung. Engelsen[28] in Christiania reported a case of three months' duration, which had previously been diagnosed as tuberculosis, until the spirochetes were found in the sputum. Under arsenical medication the recovery was soon complete.

1923. Pageniez and Ravena,[29] France reported a case of fourteen years' duration occurring in a young woman. Numerous spirochetes were found in the sputum, no clinical or radiologic changes were detected in the lungs, also in this case the arsenicals were without effect. Carulla, Besimsky and Sanguinetti in Argentina[30] reported a case of four years' duration with symptoms of chronic bronchitis before the sputum became hemorrhagic and Castellani's spirochetes were discovered. Risquez, in Caracas, Ven.,[31] reported a series of cases occurring in the course of a routine examination of two hundred patients, finding 29 per cent positive with spirochetes. He states that the disease seems to be contagious and reported antimony by the intravenous injection as the most effective treatment.

1924. Maglione and Palazzo[32] in the Argentina reported a case from which they were successful in transmitting the infection to guinea pigs. The disease proved to be contagious, rapidly spreading to other pigs. Gazette and Massias[33] in Spain reported several cases, one case in particular which did not respond to any treatment. A fusiform bacillus was present, associated with the spirochetes, in all of their cases. Jackson, U. S. A.,[34] reported a case with pseudomembranous bronchitis due to Vincent's organisms. McNeill, U. S. A.,[35] reported a case with infection of the parenchyma of the lung, indistinguishable from tuberculosis, which recovered rapidly under intravenous arsphenamine therapy. Schwarz[36] reported in the London *Lancet* six cases with typical symptoms but always associated with pyorrhea alveolaris and very few pulmonary signs. All cases recovered under arsenical treatment.

Several other writers have observed infections of this nature, including J. H. and W. B. Rothwell and W. H. Peters. From this long list one can readily observe that this is not a fanciful condition arising in the imagination of any one mind, and certainly it deserves more careful consideration than has been awarded it in the past.

All writers agree upon the appearance of the organism and in their opinion that the spirochetes are capable of producing bronchopulmonary lesions. Many believe, however, that they are a species of the ordinary spirillum found in the mouth that are capable of assuming pathogenic properties. Some think them the same as Spirocheta micro- and macrodentium; others as

a form of Vincent's spirillum as they are often associated in the lesions with a fusiform bacillus.

Castellani classifies them as a spirochete or spironema rather than a treponema. They are very actively motile, ends are accuminate; they are highly refractile, and vary in length from 5 to 25 microns and in breadth from 0.2 to 0.3 microns. Their motility is soon lost after removal from the body, when granules are formed, from which it is believed new spirochetes develop. The undulations vary in number from 2 to 16, the length of each being from 1 to 1.9 microns. The organism has no spores or flagellae and no undulating membrane. It stains poorly with ordinary analine dyes; it is gram-negative; it stains best by Fontana or Levaditi's stain; and it is fairly well stained by steaming with carbolfuchsin.

The organisms are difficult to cultivate and ordinary laboratory animals most often die from other infections which are transmitted along with the spirochetes, owing to the inability to separate the organisms. The disease has been reproduced in the monkey; it has also been successfully transmitted to guinea pigs.

SYMPTOMATOLOGY OF THE INFECTION

The Onset varies considerably; the majority of the cases reported were at first diagnosed as tuberculosis, and many had the classical symptoms of this disease such as fever, cough, profuse expectoration, with or without hemoptysis, night sweats, loss of weight, headaches, etc. Other cases had no appreciable symptoms except the spitting of blood. The chest findings also vary considerably from slight loss of resonance and diminished breath sounds to complete dullness over whole lobes.

The Sputum Findings are quite similar in all cases, the absence of the tubercle bacilli and elastic tissue and the presence of numerous short and long spiral organisms together with the ordinary bacteria of the sputum. Often an association with large numbers of fusiform bacilli is noted. This organism is often found in the normal sputum and its presence noted in many conditions. Many think it has nothing to do with the infection and is present only as a secondary invader, as are the other mouth organisms. Others place considerable importance upon these bacilli, stating that as in Vincent's angina they are but transition forms of the spirillum. There have been cases reported where the severity of the infection has been such that pulmonary gangrene resulted and death occurred. The organisms were found in large numbers in the tissues at autopsy.

Other findings, such as secondary associated anemia and occasionally gastric disturbances due, in the writer's opinion, to the swallowing of large numbers of these organisms during the attack. The teeth and tonsils are occasionally involved.

The course of the disease is usually acute, and hemoptysis is a frequent symptom. The disease may be self-limited running a course of ten days or two weeks, with a return to normal and a recurrence after a brief period. The tendency is to become chronic when the symptoms may become quiescent, the spirochetes remaining in the sputum. The lung involvement appar-

ently varies considerably, and there may be nothing more than bronchitis or a peribronchial infiltration. The areas may increase in size until lobules and even whole lobes may be involved. Pulmonary gangrene may occur.

In laboratory findings the sputum examination is by far the most important. It is necessary to use a stain that will bring out these organisms, as otherwise they are easily overlooked. The persistent absence of the tubercle bacilli and elastic tissue and the finding of numerous spiral organisms together with the ordinary bacteria of the sputum, will generally suffice to call one's attention to the possibilities of spirochetosis. An associated anemia with a fall in the number of red blood cells and hemoglobin is usually present, especially where hemoptysis takes place. The white count is usually normal or slightly elevated with an increase in the large lymphocytes and eosinophiles. Urinary findings usually negative; blood cultures persistently negative.

Treatment.—The disease is readily amenable to the proper treatment. Expectorants seem to aggravate the condition, while the arsenical compounds act almost as a specific. Such combinations as Fowler's solution with hypophosphites followed by iron and quinine will work wonders in these cases. The use of neosalvarsan intravenously is also indicated. Other symptomatic treatment, especially where considerable hemoptysis occurs, may be recommended. Antimony has been recommended, especially by intravenous injection.

Conclusions.—These cases are not common but undoubtedly they are more common than we have heretofore believed. Many think this infection is present only in the tropics or in persons coming from these regions. If more examinations were made with this diagnosis in view, however, we would find that some of our supposed tuberculous cases would undoubtedly be bronchial spirochetosis.

CASE REPORTS

I have to report, besides the two cases I saw in Colombia, S. A., three cases that I have seen in Toledo during the last seven years and two others that I did not see but of which I made the sputum examinations, and, in reviewing their histories, I believe them to be of this nature.

Two cases reported by the writer were observed in the hospital of the United Fruit Company, Santa Marta, Colombia, S. A., 1914. These were reported by letter to the medical department of the company, enclosing paper on bronchial spirochetosis for publication in the London *Lancet.*

CASE 1.—Adult male, Colombian, apparent age thirty years. Family history negative.

Past History.—Stated that he had never been sick, not even had he suffered from malaria, so prevalent in that region.

Present Illness.—Two months previous to entering the hospital he had been exposed to several severe wettings from tropical rains and had slept out in the open; he had contracted a cold on the chest and a slight cough. This continued without fever for about ten days, when he had a chill and was sent to the hospital for treatment. Diagnosis, tuberculosis. Complains now of headache, fever, chills and cough with profuse expectoration of slimy sputum. No blood in sputum.

Examination showed *head* normal; some sore throat and injection of tonsils; teeth not bad; cervical glands palpable, as were the inguinal and subaxillary glands. *Chest* well developed, expansion good and equal on both sides. Diminished resonance over both apices, most marked on right side. No change in tactile or vocal fremitus. Roughened breath

sounds were heard over both bronchi, soft crepitant râles were heard over both apices, more marked on right side anteriorly. *Heart:* Slight accentuation of second pulmonary sound, otherwise negative. *Abdomen:* Liver normal. Speen slightly enlarged and tender.

Laboratory Examination showed Wassermann negative, red blood count normal, hemoglobin 90 per cent, white blood count slight increase to 8,000, differential smear showed an increase in mononuclear cells and negative for malaria or other parasites.

Sputum, stained with carbolfuchsin, methylene blue, was negative for tubercle bacilli but showed the presence of large numbers of large spiral organisms stained slightly pink. Another stain, made by simply staining with carbolfuchsin and washing in water, showed the organisms very well. They were by far the predominating organisms present. No fusiform bacilli were present, the other organisms found being staphylococci and streptococci and a short gram-negative bacillus, probably Pfeiffer.

Course of the Infection.—The patient was given an expectorant cough mixture with an immediate aggravation of the symptoms. The fever continued for about six days showing slight remissions, but, after patient was given Fowler's solution with hypophosphites, the cough and sputum diminished and fever gradually fell to normal. After about ten days' treatment no spirochetes were found in the sputum.

Routine examinations were now made on all natives entering the hospital suffering from bronchitis and soon a second almost identical case was encountered.

CASE 2.—A male, Colombian, apparently forty years of age.

History of the Infection.—The patient had been subject to similar attacks, generally following exposure and always starting with symptoms of a cold. Chills and fever were always present, with the expectoration of considerable foul smelling mucoid sputum, and no hemoptysis.

Examination.—The *neck* showed enlargement of the cervical lymph glands. The *chest* revealed some dullness over the right upper lobe anteriorly with moist râles over the same area. Heart sounds were normal. The *abdomen* showed no abnormal findings.

Laboratory Findings.—Blood, red count, 4,500,000; white count, 9,500; hemoglobin 80 per cent. *Differential count showed an increase in the large mononuclear lymphocytes.* No abnormal changes in the red cells, no malarial parasites were found. *Sputum* showed numerous spiral organisms. Stained by steaming with carbolfuchsin; no tubercle bacilli were present. A few short bacilli were found. Dark-field examination showed these spirochetes to be actively motile, resembling the mouth spirilli.

Treatment.—The patient was put on Fowler's solution and hypophosphites; the symptoms rapidly abated and returned to normal in one week.

The following cases were observed by the writer in Toledo, Ohio.

CASE 1.—Mr. B. C., ex-soldier, age twenty-seven years.

Family History.—Father alive and well. Mother had been sick for three years, had pneumonia with delayed resolution, abscess of the lung and empyema. Two brothers alive and well. No tuberculosis in the family.

Past History.—Has had ordinary diseases of childhood, including diphtheria. No other serious illnesses; has occasional attacks of cold and tonsillitis.

Present Complaint.—Pain in the chest, cough, expectoration of large quantities of blood. Had been well up until a few months before leaving the service, when he had a similar attack with pain in the chest, cough and hemorrhage. Was confined in a hospital in camp, recovered rapidly. At this time a diagnosis was made of pulmonary tuberculosis, although at no time were any tubercle bacilli found in the sputum. After dismissal from the service he had another attack with hemorrhage as the main symptom. I first saw this patient May 1, 1919; he was expectorating considerable blood, spitting up probably a pint altogether. With proper treatment bleeding stopped and examination was made the next day.

Examination showed a well nourished and well developed male; the head was generally negative. The throat and tonsils were inflamed. The teeth in good condition; the cervical glands were palpable. *Chest well developed,* expansion normal, no sinking in of the supra-

clavicular spaces. Percussion right lung normal, left lung showed an area of dullness over the upper lobe below the clavicle, some dullness being noted posteriorly over base. Some râles were heard over these areas. No other important pulmonary findings. Heart was negative except for slight accentuation of second pulmonary sound. Abdomen and extremities were normal. The pulse was 80, respiration 22, temperature 99 degrees.

Laboratory Findings.—Throat cultures were negative except for ordinary organisms. Urine normal. Sputum examination showed blood cells, pus cells, mucous shreds and bacteria. Fontana stained smears showed numerous spiral organisms. No tubercle bacilli or elastic tissue was found.

Treatment.—Patient was confined to bed; was given Fowler's solution and hypophosphites with iron and quinine. He improved rapidly under this treatment, and after one month the lung findings had cleared almost entirely. No spirochetes were found in the sputum.

Subsequent History.—I was able to communicate with this patient for several years, all of which time he has remained well and has had no more attacks.

Case 2.—Mr. J. W., age nineteen years.

Family History.—Nothing of importance in the family history; no tuberculosis.

Past History.—Ordinary diseases of childhood; no other serious illness.

Present Illness.—Started two months ago with sore throat, huskiness of the voice, cough, developed chills and fever, with headache, backache and gastric disturbances. Ran a fever for ten days, became better but cough and huskiness of the voice persisted. Has spit up quite a bit of dark brown material.

Examination showed head generally negative; teeth were in poor condition, throat inflamed, and the cervical glands prominent. Chest showed diminished resonance and moist râles over both apices, the right apex being more involved. Heart sounds were normal, abdomen and extremities were negative. Temperature 99.6 degrees, pulse 84, respiration 22.

Laboratory Findings.—Throat cultures were negative except for ordinary organisms. Urine examination was negative. *Sputum* examination negative for tubercle bacilli, positive for staphylococci, diplococci and streptococci; a few bacilli and numerous spirochetes were found.

Treatment.—Patient given arsenical treatment, later iron and quinine, with rapid improvement and apparent complete recovery. No subsequent attacks occurred.

Case 3.—Mr. S. A. S., age fifty-eight years.

Family History.—Nothing of importance in the family history.

Past History.—Had ordinary diseases of childhood and pleural pneumonia on right side. Had an attack of influenza several years ago from which he never fully recovered. Has had a chronic nasal infection. Has been suffering from attacks of influenza for several years, being confined to bed a week or more at a time. Has had herpes zoster of more or less chronic nature. Several months previous to this examination patient had an attack of pain in the chest with fever and cough, with increased expectoration lasting about ten days. Recovered somewhat, but cough persisted and he still expectorates considerable slightly blood-tinged sputum. No night sweats have occurred at any time.

Examination showed a well developed adult male, head negative except for a few blotches of herpes on the left cheek. The teeth were in fair condition, the throat and pharynx reddened. No sinus involvement was found. Chest was fairly well developed, resonance diminished somewhat over the right middle and upper lobes, no râles were heard. The right apex somewhat dull and areas of crepitation were heard. Heart, a soft systolic bruit was heard over the aortic area and the heart seemed slightly enlarged. Abdomen and extremities were negative. Pulse 90, temperature 100 degrees, and respiration 20.

Laboratory Findings.—Urine examination was negative. Red blood count 4,800,000; white blood count 8,500; hemoglobin 85 per cent. Sputum examination showed pus cells and numerous bacteria, including staphylococci and streptococci, tetrads and Spirocheta bronchialis. Cultures were attempted to try to cultivate this spirochete, but the efforts were not successful.

Treatment.—Arsenical medications with iron, quinine and hypophosphites caused a clearing of the sputum and some improvement. We lost track of this patient, and the ultimate outcome is not known.

CASE 4.—Mr. F. W., age twenty-eight years, ex-soldier. Original diagnosis, pulmonary tuberculosis.

Family History.—Nothing of importance; negative for tuberculosis.

Past History.—Always well as a child and boy, except for ordinary childhood diseases and frequent attacks of tonsillitis. Tonsils were removed in 1915.

Present Complaint.—Nervousness, headaches, loss of weight, cough and numerous hemorrhages from the lungs. Has had attacks of fever and has frequent night sweats. These symptoms are of about one year's duration.

Examination.—Patient shows some evidence of loss of weight and is rather pale and anemic in appearance. Head: right ear is discharging and has total deafness on this side; other ear normal. Eyes are normal. Teeth are in bad condition. The throat is red and inflamed. The cervical glands are easily palpable on both sides. The chest shows poor expansion, especially on the right side. The supraclavicular spaces are sunken and the intercostal spaces are depressed. There is a dullness over the right lung with bronchial breathing and crepitant râles. The left lung shows scattered areas of dullness with crepitant râles. The heart is slightly enlarged; no murmurs. The abdomen and extremities are negative. Pulse 70, temperature 99 degrees, respiration 20.

Laboratory Findings.—Many examinations of the sputum were made. Pus, epithelial cells, mucous shreds and numerous bacteria, including all the forms of cocci, together with numerous bacilli and spirilli. At no time were tubercle bacilli found. The spirilli were only faintly observed with ordinary staining methods, but the Fontana method showed them to be present in large numbers in every field. The urine was normal.

Treatment.—The patient was not treated by the writer, and to my knowledge no arsenical medication was given. This case remained in the hospital for about one week; was not confined to bed; had some cough, no hemoptysis. This case was reported to the physician in charge as probably pulmonary tuberculosis with a secondary spirochetosis.

CASE 5.—Mr. C. S., age twenty-seven years, ex-soldier. Original diagnosis, pulmonary tuberculosis.

Family History.—Negative except that the grandfather on mother's side probably died from tuberculosis.

Past History.—Has had the ordinary diseases of childhood. Had typhoid fever and suffered from asthma while a boy. No serious illnesses since then until three months after discharge from the army. Smokes many cigarettes daily. Venereal history negative.

Present Complaint.—General weakness, cough and expectoration of a greenish sputum, loss of weight, some fever in the afternoon. Has all the classical symptoms of tuberculosis. Duration about two years; has occasional night sweats. Has not noticed any blood in the sputum.

Examination showed average development, fair nutrition, slight anemia. Head is negative, except that the teeth are in poor condition and the tonsils and pharynx are inflamed. The cervical glands are palpable on both sides of the neck. The chest is fairly well developed with some depression of the supraclavicular spaces, expansion fairly well equal on both sides, breathing regular, not hurried or dyspneic. Breath sounds are clear anteriorly with some few râles heard posteriorly over both bases, especially at the end of deep inspiration. Heart normal. Genitalia negative. Extremities negative. Pulse 72, temperature 99 degrees, respiration 20.

Laboratory Findings.—Daily sputum examinations were all negative for tubercle bacilli, but contained staphylococci, streptococci and diplococci. Many bacilli and numerous spirilli were found. These spirilli were of the Castellani type and stained only with the silver nitrate method. No fusiform bacilli were noted in the sputum. Red blood count 3,286,000; white count 10,860; hemoglobin 65 per cent. Differential count: polys 65 per cent, large lymphocytes 20 per cent, small lymphocytes 12 per cent, transitionals 3 per cent.

Subsequent History.—The temperature while in the hospital was subnormal, except for

one elevation to 100.8 degrees, after which it rapidly returned to subnormal. The pulse ranged from 60 to 90 per minute. Respiration ran around 20. The patient was not confined to bed and was discharged after three days. The diagnosis of bronchial spirochetosis was submitted to the physician in charge.

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DISCUSSION

Dr. F. H. Lamb.—I would just like to ask two questions about spirochetosis. In this condition has Dr. Ramsey observed an increase in size and an apparent increase in the ability of the organisms to take up stain in cases of acute Vincent's angina and why do acute attacks of Vincent's angina occasionally develop in cases of chronic syphilis during the course of administration of salvarsan? It is held by some that salvarsan and neosalvarsan are specific remedies for Vincent's angina.

Dr. A. H. Schade.—I had two cases which the clinicians had diagnosed tuberculosis. I could not find tubercle bacilli in the smear. We were able to demonstrate definitely the spirochetes. They both made rapid recovery and are perfectly healthy at this time, after three years. One of them had watery, bloody sputum; the other's was just blood streaked. They had a typical picture of tuberculosis. One case was treated with Fowler's solution and the other with neoarsphenamine. There was no choice between the two remedies in these two cases.

Dr. H. J. Nichols.—The subject deserves consideration by the Society because there seems to be a very definite entity which, as Dr. Ramsey has said, is due to spirochetes. There may, of course, be some underlying disease which predisposes to spirochetosis in the bronchi. During the epidemic of influenza there was definite evidence that in some cases of secondary pneumonia there were a great many spirochetes in the lungs. I think this condition should be watched and apparently it can be terminated promptly with the arsphenamine.

The cases we have seen have been characterized by serous and bloody expectoration. This is a very timely paper.

Dr. O. Lowy.—I would like to ask Dr. Ramsey whether or not he used gentian-violet in staining. I have used it in all my stains, and I have found a great deal of Vincent's angina.

Dr. Thomas L. Ramsey (closing).—Regarding the staining characteristics of the organism: the spirillum of Vincent's angina stains fairly well with all ordinary aniline dyes and can be seen readily in smears stained by Loeffler's methylene blue. The Spirocheta bronchialis can only be stained, so far as is known, by silver nitrate methods and by steaming carbolfuchsin. I am of the opinion that bronchial spirochetosis is a distinct entity. In regard to attacks or exacerbations of attacks of Vincent's angina brought on by administration of the salvarsan, I have never seen this occur and cannot see any possible connection with salvarsan administration and Vincent's angina. Exacerbations of inflammation of the throat may occur in syphilitic throats immediately following salvarsan administration; this is then followed by almost immediate improvement of the condition.

PNEUMOCOCCUS ANTIBODY SOLUTION*

By F. M. Huntoon, M.D., Glenolden, Pa.

SEPARATION of the immune body from the other serum constituents has been an ideal since the discovery that so-called immune serums contained a substance which, when brought into contact with the homologous antigens, caused certain effects which we have called antibody action.

Such separation was desirable not only for practical reasons, therapeutic usage, but also for the possibility that it might lead to an identification of this antibody substance, which shows in its specific affinities one of the most remarkable phenomenons of nature.

A serum may be regarded as being a colloidal solution containing water, salts, and certain protein substances, which are designated as euglobulins, pseudoglobulins and albumins. An immune serum is a similar mixture plus the antibody substance. In fact we have some evidence that antibody really acts as a foreign body and is eliminated as such.

If, to an immune serum, a ponderable homologous antigen (bacteria) is added, a combination promptly takes place between the antigen and the antibody; the so-called sensitization process or the absorption phenomenon. If the sensitized antigen is removed from the serum by centrifugation or otherwise, and the traces of serum which remain are removed by washing with salt solution, we have remaining a combination of bacteria and antibody free from all gross evidences of the original protein environment of the antibody.

In other words, the antibody has been removed from the other serum constituents. If means could be found to separate the attached antibody from its combination with the antigen, an antibody solution could be obtained free

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From Mulford Biological Laboratories.

from the protein material which accompanies antibody in immune serum. Such a procedure has been found and utilized in the preparation of the pneumococcus antibody solution. Briefly the procedure is as follows:

To a polyvalent pneumococcus serum containing antibody against types I, II, and III is added pneumococci of the three types; this is allowed to sensitize in the ice box. The organisms are then removed by centrifugation and are washed twice with ice cold salt solution. They are then emulsified in salt solution and an amount of alkali added in proportion to the number of organisms present. After allowing it to stand, usually, overnight, the material is again centrifuged and the supernatant fluid is removed and saved. On testing this fluid it is found to contain both agglutinins and the protective pneumococcus antibody. The solutions at this stage are somewhat opalescent, because of dissolved bacterial proteins. By various procedures, including storages at low temperature and careful changes in the P_H, most of this material can be removed without materially affecting the antibody contents. The solutions are finally cleared and rendered sterile by passing through Berkfeld filters.

We now have a clear, water colored solution which exhibits all the antibody action of the original serum. That it contains no large amount of serum, however, is shown by the attempt to sensitize guinea pigs with such solutions. When subsequently tested by the intravenous injection of horse serum, approximately 50 per cent of such animals fail to show any evidence of anaphylaxis. This indicates that any serum protein present must exist in very small amounts. Further, in the treatment of over 800 cases of pneumonia with this material we have not in a single instance seen evidences of serum sickness or anaphylaxis, although it has been given to those known to be sensitive to horse serum.

We have in our experience prepared solutions which failed to give any of the known chemical tests for proteins, but still retained antibody action. We have determined, moreover, that antibody under these conditions resists certain deleterious influences to a greater extent than the antibody in the serum. For instance, the influence of incubator temperature reduces the antibody contents little or none over a period of a month, in fact even the addition of trypsin brings no apparent change. Either antibody is not a protein substance susceptible to trypsin or the action of the trypsin is blocked by some other constituents of the solution. Such antibody solutions are not entirely precipitable by ammonium sulphate even when high as 90 per cent saturation is employed.

Antibody, when attached to antigen, is not destroyed by long immersion in absolute alcohol. On the other hand the antibody in such solution is susceptible to the influence of preservative, such as phenol and tricresol. We can say that the tests available tend to show antibody to be a nonprotein substance, but this is far from being proved.

In the original therapeutic work which was undertaken with these solutions, the administration was by the intravenous route and this administration was almost invariably accompanied by a reaction simulating the so-called foreign protein shock (chill, fever and sweating). Such reactions, while

occasionally alarming, were seldom dangerous. In hospital practice, they were not regarded too seriously. The question arose at this time as to the causation of the reaction and its influences on the therapeutic results obtained. There is no doubt that, combined, with the antibody action, it results in many dramatic cures. Following the observation that antibody injection under the skin of rabbits rapidly appeared in the circulation, a certain number of early cases of pneumonia were treated by the subcutaneous route. The results not being as favorable as was hoped, although the reaction was absent, led to further experimental investigation and brought about the discovery that the antibody in such solutions when placed under the skin of the human subject did not appear in the blood stream in any large quantity. This is not true in all cases, particularly in children. It caused, however, the abandoning of the subcutaneous route.

Investigations were undertaken to discover if it was possible to so prepare this solution that the intravenous injection would not be followed by a reaction. The solution of this problem apparently consists in using the greatest care to avoid any extraneous bacterial contamination at any stage of the process. The entire procedure is now conducted at ice box temperature and the majority of the antibody solutions so prepared have been free from the shock factor. The clinical trial of such shockless antibody solution has shown the same beneficial effect in early cases that were noted with the earlier solutions but without the dramatic features of the sharp rising temperature with the immediate fall to or about normal. The temperature falls gradually over periods of two to three days with a coincident improvement in all the symptoms. A very recent development has been the discovery of a rather simple method by which such solutions may be concentrated. It has been found possible to produce a concentration of as much as forty times. While this development is still very much in the experimental state, it is hoped that instead of administering the rather formidable dose of 50 to 100 c.c. a similar or larger dose of antibody may be given in a 5 to 10 c.c. dose.

Six years of experience indicates that the preparation of such solutions is the route by which the ultimate nature of antibody will be determined and that it offers at present the best method for the therapeutic use of specific immune body.

DISCUSSION

Dr. John A. Kolmer.—This interesting summary of Dr. Huntoon's work is now open for discussion, and I may state that the method developed constitutes a useful start in the perfection of a technic for the dissociation of antibody in a pure state for the highly desirable studies on the chemistry of antibodies. During the past year Dr. Uchida in my laboratory has been interested in developing the method for the isolation of complement-fixation antibody for chemical studies.

Dr. Robert A. Kilduffe.—I should like to ask what is the dosage of this preparation. I was also much interested in the medium mentioned for the cultivation of the pneumococcus, and I should like to know whether its preparation and formula have been published.

Dr. A. C. Abbott.—I am more or less familiar with Dr. Huntoon's work. Manifestly, it is still in the experimental stage, and by the complexity of its nature it originates questions that must be more convincingly answered. For instance, one such question,

at least, relates to the interpretation of the action of the alkali used. It is difficult for me to believe that that action is limited to disengaging the antibody from the antigen, the bacteria. I cannot avoid an opinion that in addition to that function there must be a direct action upon either the bacteria themselves or the antibody, or both. That question, however, if of importance, can safely be left to Dr. Huntoon for answer.

Dr. David H. Bergey.—Dr. Huntoon's work is of great interest, because fundamentally it serves to extend the clinician's methods of treatment. The desirability of placing in their hands an antibody solution is so great that it is not surprising that many clinicians have continued to use this solution in spite of undesirable reactions having accompanied an earlier preparation. The elimination of this has been a large part of the object in perfecting his solution.

Dr. Robert A. Keilty.—We have been using this from the beginning. Our results have justified the continuance of it. It is rather expensive. What are the future possibilities of a still further reduction in price?

Dr. F. M. Huntoon (closing).—The first question I believe was about a precipitate forming in the solution. Certain of these solutions will after a time form a precipitate which goes into solution again when heated to body temperature.

The question of medium I can answer very quickly. This formula has been published in the Journal of the Society of Experimental Biology.

The question of dosage was originally based on the amount of antipneumococcus serum that had been used. During the first two years, Dr. Cecil did four hundred cases; he used large doses. Dr. Conner at the New York Hospital gave comparatively smaller doses, 25 c.c. and 50 c.c., the second dose of 50 c.c. to men. Dr. Conner's results were better from a statistical viewpoint than the others. Men this year like to use 100 c.c. doses and they are generally perfectly safe. If no results are obtained with three or four doses, you might as well stop.

The question of expense: that depends entirely on volume; the cost to the consumer will automatically drop as the production rises. Experimentation on this scale costs a great deal.

Now the question as to whether these solutions are pure antibody or not. No, far from it. We have produced highly potent material which gave none of the chemical tests for proteins, but that material still contains somewhere around 3 mg. of nitrogen per hundred cubic centimeters. The mere statement that it gives none of the chemical tests for protein does not mean that the proteins are not there. My personal opinion is that the antibody is still combined with antigen, that when you take off the antibody a small portion of the antigen splits off with it. It is impossible to prepare it in the pure substance. The fact that it is combined with antigen does not impair its reaction in the least. I doubt very much that there is any antibody in the solution that is in a free state. The question of obtaining absolutely pure antibody, I doubt if it is possible.

THE PROBABLE RÔLE OF THE BACTERIOPHAGE IN STREPTOCOCCUS INFECTIONS*

By L. O. Dutton, M.S., Memphis, Tenn.

THE literature concerning the bacteriophage has few references to the phenomenon of transmissible lysis of the streptococci. D'Herelle[1] mentions that streptococci have been lysed, and McKinley[2] reports that he has adapted a phage that was secured from d'Herelle to the streptococcus. McKinley[3] also reports the successful treatment of a streptococcus lung abscess with the bacteriophage. Aside from these meager details there is little that is enlightening concerning the bacteriophage as a factor in streptococcus infections. The literature also leaves one with the impression that the bacteriophage is not readily isolated from the blood stream and, if it is found there, it remains for a very short period only. The data detailed here is offered because it suggests an interesting phase in the understanding of streptococcus infections, as well as speculations of interest as to the nature and distribution of bacteriophage strains active for these organisms.

The isolation of a bacteriophage active for streptococci reported here came about rather as an accident. A blood culture was taken from a patient who had been operated upon two days previously for a mastoid infection. On this day the temperature of the patient rose to 105° F. Ten c.c. of blood were delivered into a flask containing 200 c.c. of veal infusion dextrose broth, P_H 7.4, and 1 c.c. of blood was delivered into each of five tubes containing 10 c.c. of dextrose agar, P_H 7.2 mixed, and plated. The following morning, fifteen hours after culturing, the flask and plates were examined. There was a cloud in the flask but there were no colonies on the plates. Smears from the flask revealed gram-positive cocci in pairs and in short chains. Transplants were made from the flask to blood agar and to dextrose agar. The cultures were then incubated for another twenty-four hours. At the end of this incubation period, there were still no colonies on the agar plates, nor was there growth on the plants from the flask. A second smear from the flask at this time failed to reveal any bacterial cells at all in spite of long search. After another twenty-four hours growth, the transplants from the flask on blood agar had developed a few very peculiar colonies. They were large, mucoid, and transparent, with a greenish color. Smears of them revealed a streptococcus-like organism with a great many atypical cells. Smears from the original flask at this time failed to show organisms.

An hypothesis to explain the unusual findings was sought by assuming the presence of a bacteriophageous lytic principle in the blood of the patient or carried in the strain of streptococcus itself. Subsequent study of the culture seemed to substantiate the latter assumption. The behavior of the patient was

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interesting in the light of such cultural findings. After a short period, twenty-four hours, of high temperature, the general condition of the patient became improved, the temperature and pulse rate fell to normal, and in the course of four days, the patient was discharged from the hospital.

A report of the detailed study of the culture will appear in the near future. It is sufficient to say here that, so far as the particular strain of streptococcus involved in this study, transmissible lysis was never obtained. By washing the entire growth from an agar slant into a flask of plain infusion broth, however, a definite and heavy growth took place in eighteen hours that became completely lysed in twenty-four to thirty hours. This phenomenon could be obtained as long as growth was maintained on agar. The bacteriophage became so lytic in its activity, however, that, after the course of two months transplanting, the cultures failed to grow. This phage is to be designated as phage "H" hereafter.

In the absence of transmissible lysis, and in view of our incomplete knowledge of streptococcus cultures mixed with a bacteriophage, it was impossible to conclude that the bacteriophage was the factor responsible for the lysis of the culture. We have since been able to produce experimental transmissible lysis of other strains of streptococci, however, and conclude that we are dealing with a bacteriophage active for these organisms.

Assuming the correctness of d'Herelle's hypothesis as to the rôle of the bacteriophage in recovery from disease, it becomes logical to conclude that the bacteriophage was the factor responsible for the recovery of this patient. The therapeutic use of this phage then becomes the next obvious step.

A patient having a precipitous delivery developed thirty hours after delivery a temperature of 105° F. A blood culture taken several hours later showed, at the end of eighteen hours' incubation, a heavy growth of a streptococcus of the hemolytic type. The general condition of the patient at this time was very grave. One c.c. of phage "H" was injected intravenously. Following the injection there was a rise in temperature of .6° F. There was no general reaction, however. After two hours the temperature began to recede gradually and the patient felt better. The lowest point of the temperature curve for the day, however, was 102.4° F. The following morning, a second 1 c.c. of phage was given intravenously. This was just twenty-four hours after the initial injection. Four hours later a second blood culture was taken and, with the same needle, a third 1 c.c. of phage was given. The patient's temperature persisted at a high level, however, and twelve hours after the third dose of phage 25 c.c. of 1 per cent gentian-violet was given. For the next twenty-four hours the temperature curve was constantly at a high level, and a second dose of gentian-violet was given. Twelve hours after this last dose and fifty-two hours after the initial phage dose, the temperature was normal, and remained so except for one high peak on the third day following. Otherwise the patient went to a rapid and complete recovery. The second blood culture, taken before gentian-violet was given, was sterile after five days incubation.

An analysis of this case leaves us somewhat in doubt as to the relative parts played in the recovery by the phage and gentian-violet. Experiments

were done, however, that are interesting in the explanation of this point. To begin with the organisms isolated from the first blood culture were subjected to the action of phage "H" in a series of passages. It was found that at the eighth passage the tube containing the phage was completely lysed, while the control tube contained a heavy growth. This established the fact that the organism was sensitive to the action of phage "H." The next experiment was to employ a filtrate of the first and second blood cultures as a starting point for a possible phage and subject the organisms to a series of passages. In this experiment, the passages of the second culture showed lysis on the fourth passage, while the passages of the first culture failed to show lysis even after 15 passages. This indicates that there was no lytic principle in the patient's blood at the onset of the disease and that there was such a principle present after innoculation of a bacteriophage. Controls of this experiment were a series of passages from a sterile blood culture of another patient and a series of passages without any initial inoculation of a phagic or probable phagic substance. That is, a normal culture was filtered and passed into a second culture. These controls established that the serum of the blood had no part in the lysing of the organisms and that the organism was carrying no phage mixed with it. This chain of evidence is nearly conclusive that the lytic principle was the factor responsible in a large measure for the recovery of the patient. These experiments are tabulated under Exper. I.

A second case was chosen to try the therapeutic value of the phage. This was a hemolytic streptococcus infection of about four weeks' duration. The blood culture was positive and the patient had many abscesses in various parts of the body. Those that were visible were in the muscle septa. The patient was given 1 c.c. of phage "H" intravenously. On the next day the patient was not improved. A second blood culture was taken. This culture proved to be positive and the patient died on the fourth day following the phage injection with the symptoms of an embolism.

The experiments done on the previous cultures were repeated with interesting results.

In this series of tests we were not able to obtain lysis of the organisms under any conditions. The passage of the phage that had been given the patient always remained cloudy as did the passage of the blood culture filtrates. Thus it was demonstrated that the organism responsible for the condition of the second patient was resistant to the phage, and that no lytic agent was in the patient's blood. These are detailed under Experiment II.

Without giving the details of other cases it is sufficient to say that the phage has been used intravenously on three other definitely proved streptococcus blood stream infections. Of these three, two patients recovered, one following an acute arthritis with hemolytic streptococci in the blood stream, and one following a septic hemolytic streptococcus infection after a mastoid primary infection. The third patient died after having received the phage intravenously during the end stage of a streptococcus viridans endocarditis. The injections were given as in the successful case detailed above and the experimental results obtained were identical with those given in detail.

The technic of the experiments was as follows: Veal infusion broth ad-

EXPERIMENT I

SERIES	STRAIN OF STREPTOCOCCUS	FILTRATE	PASSAGES 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	428	H	C	C	C	C	C	C	C	L	L	L	L	L	L	L	L	L	L	L	L
2	428	First Blood Culture	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
3	428	Second Blood Culture	C	C	C	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
4	428	Auto Filtrate	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
5	428	Sterile Blood Culture	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
6	428	None	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C

Strain 428 isolated from patient who recovered following injection of phage.
Filtrate H., lysed culture of strain isolated from patient who recovered spontaneously.
Auto filtrate, filtrate of a normal broth culture of strain 428.
"C" indicates normal cloud after 24 hours' incubation.
"L" indicates clear tube after 24 hours' incubation.

EXPERIMENT II

SERIES	ORGANISM	FILTRATE	PASSAGES 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	440	H	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
2	440	First Blood Culture	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
3	440	Second Blood Culture	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
4	440	Auto Filtrate	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
5	440	None	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C

H Filtrate, lysed culture of organism 428 after addition of H phage.
"C" indicates normal clouds after twenty-four hours' incubation.

justed to P_H 8 was tubed in 10 c.c. quantities, and, after isolation of an organism, a few transplants through this medium resulted in characteristic streptococcus growth. Most of the strains studied in this medium have given consistent and abundant growth from the very start. It is important that carbohydrates are not used in the medium, as the organisms ferment the sugar to result in a shift of the P_H to the acid side, and it is necessary that the medium remain alkaline to obtain phagic activity. Various methods of utilizing sugars as growth stimulators with calcium carbonate or buffers to control the P_H were discarded in favor of the more constant veal infusion plain broth. When the organisms showed a good growth in this medium a tube was inoculated, and three hours later a second tube was inoculated from the first. This second tube was then incubated for a three-hour period, and a known or suspected phage was added to it. In general 1 c.c. quantities of the phage were used. These tubes were incubated at 37° C. for twenty-four hours, with observation being made at various intervals. There was generally a cloud in all the tubes at the end of nine to twelve hours after inoculation. In the tubes that exhibited lysis of the organisms there remained only a slight sediment at the end of twenty-four hours, while in the control tubes and the tubes showing no lysis there was a marked cloud at this period. At the end of this twenty-four-hour incubation period the cultures were filtered through a Mandler filter. The No. 5 size was found convenient for the small quantities of media used. One c.c. of the filtrite was added to a culture planted as described above from a three-hour culture. This procedure was repeated for the successive passages.

The necessity of inoculating tubes from three-hour broth cultures, and of adding the filtrates to three-hour cultures was recognized after experiments concerning these points. It was found that lysis was not obtained when the filtrates were added to cultures that were older than three hours, and it was found that the three-hour age was best from which to make the inoculations. It has not yet been determined whether the essential factor in this step is the age of the cells that were inoculated or the decreased number of cells that would result from inoculations of such young cultures.

SUMMARY

In surveying these three cases we find that in the initial one a streptococcus blood stream infection occurs that recovers spontaneously without treatment. On study of the organism isolated from this case a lytic principle was obtained that would lyse strains of streptococci. In the second case a blood stream streptococcus infection occurred that was promptly overcome when the phage that was secured from case No. 1 was injected. This was followed by a recovery of the patient, and also, experimentally, a lytic principle was found to be absent from the initial positive blood culture, while from the second sterile culture a phagic substance was obtained that produced lysis of the streptococci isolated from the patient. Case No. 3 was found to have a streptococcus blood stream infection. After administration of the bacteriophage the blood stream still contained the organisms and the patient died. Experiments with the cultures obtained in this case failed to reveal a phagic substance either before or after the administration of the phage. Nor was it possible to obtain lysis of

the strain of streptococcus that was isolated from this case with the phage that was used. In other words the organism present in the first two cases was susceptible to the bacteriophage and the patient recovered, while the organism in the last case was not susceptible to the phage and the patient died.

CONCLUSION

These facts are all in accord with the hypothesis d'Herelle has advanced concerning the rôle of the bacteriophage in infection and immunity.

REFERENCES

[1]D'Herelle: Immunity in Natural Infectious Diseases, English edition, 1924, p. 297.
[2]McKinley: Notes on d'Herelle Phenomenon, Jour. Lab. and Clin. Med., viii, 311.
[3]McKinley: Bacteriophage in Treatment of Infections, Arch. Int. Med., xxxii, 899.

LABORATORY METHODS

A TIME- AND LABOR-SAVING PHOTOGRAPHIC APPARATUS*

By Joseph B. Homan, Cincinnati, Ohio

FIG. 1. Apparatus used in making lantern slide, negatives from books, photographs, and charts. Photographs all kinds of medical specimens, instruments, charts and any other objects conveniently laid out flat. With three lenses, 6, 10 and 14 inch focus, enlargements can be made or a 36 inch chart can be reduced to 8 × 10 inches or smaller. Apparatus is very speedy, taking from twenty to twenty-five pictures from books in one hour.

Description.—Made mostly of 1 inch I. D. pipe.

Vibration is overcome by braces. Eight 1 inch flanges in wall and ceiling and 6 lag expansion bolts in floor.

Two stands and a pipe ladder to each stand.

When the higher stand is used the lower stand can be swung out of the way, thus enabling the complete board to be used.

Thirteen feet high and takes up less than eight feet square of floor space.

No. 1.—The board, 5 feet by 36 inches, grooved to take light angles. This board is mobile, moving either east or west, north or south, kept parallel with the camera by a device under the board. These motions are obtained by the use of levers, No. 4 and No. 5, which can be reached from either stand, and *while looking onto the ground glass of the camera.* Lever 4 swings to one side when upper stand is used.

The board is laid off with center and parallel lines.

An object can be centered in five seconds.

No. 2.—Light angles; each contains 5 100-watt bowl frosted incandescent lights, each light having a separate switch.

Each angle can be moved forward or back on steel shoes, which slide in grooves on board.

Each angle can be moved up or down on rods and set at the proper height.

The lights are on a mobile board which can be moved in and out inside the angles. Each angle has three different motions to adjust light to insure exactly the proper angle of light.

No. 3.—Camera, Folmer and Schwing enlarging, reducing and copying camera with a five foot bellows extension.

The camera is held perfectly rigid, by a lever and set screw, from the upper stand.

An adjusting screw permits a motion of eighteen inches up or down. If

*From the Department of Medical Art, College of Medicine, University of Cincinnati.
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this is not enough the set screw is loosened and the complete camera is moved up or down on the pipe slide by the drum No. 6 and a counter weight or, as the lens is usually used in the middle compartment which is movable, there is another means of racking the lens up or down.

For the final focusing we have the micrometer focusing screw. There are four different means of securing a focus.

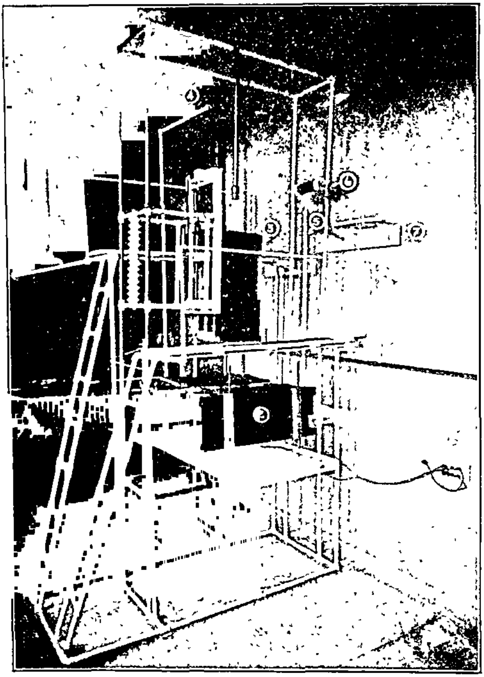

Fig 1.

Nos. 4 and *5.—Levers* which pull the picture to a center on the ground glass in five seconds. They have a point of leverage which makes them very easy to move.

Centering the picture is done while looking onto the ground glass, it being unnecessary to go down the ladder to adjust the book or picture.

No. 6.—Drum used in winding camera up or down.

No. 7.—Shelf upon which unexposed and exposed plates are put.

No. 8.—Switch to control lights.

The apparatus has a wooden rod suspended alongside of camera on which are the various lens distances and exposure time for various plates.

Fig. 2 shows detail of light angles and layout on board.

No. 1.—Side lever.

No. 2.—Light angle showing steel shoes and how lights are focused by aid of pins inside the angles.

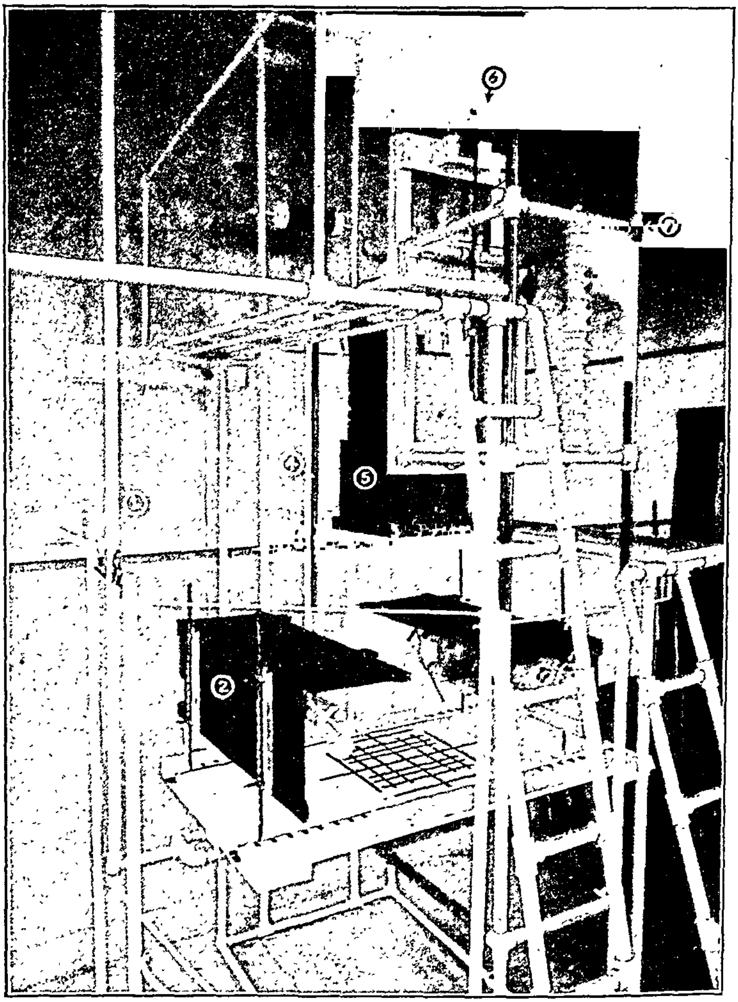

Fig. 2.

No. 3.—Back lever.

No. 4.—Wooden rod with exposure time and size.

No. 5.—Camera.

No. 6.—Adjusting screw for moving camera up and down.

No. 7.—Slide which permits complete camera to move up and down by aid of drum and counter weight.

Fig. 3 shows detail of levers.

No. 1.—Counter weight for camera.

No. 2.—Joints on which the movement of board depends.

Fig. 3.

No. 3.—Point of leverage, showing pulley which enables lever to ride nore easily.

No. 4.—Wooden rod with exposure time and size.

TWO CONVENIENT FORMS OF APPARATUS FOR MICROBLOOD AND MICROURINE ANALYSIS*

BY F. C. KOCH, PH.D., CHICAGO, ILL.

THE two devices described below have been found very convenient and reliable for individual as well as for class use in blood and urine analysis as well as for other purposes. The first is an improved distilling tube for micro-Kjeldahl or microurea distillations, and the second is a convenient device for rapidly and accurately measuring off various reagents.

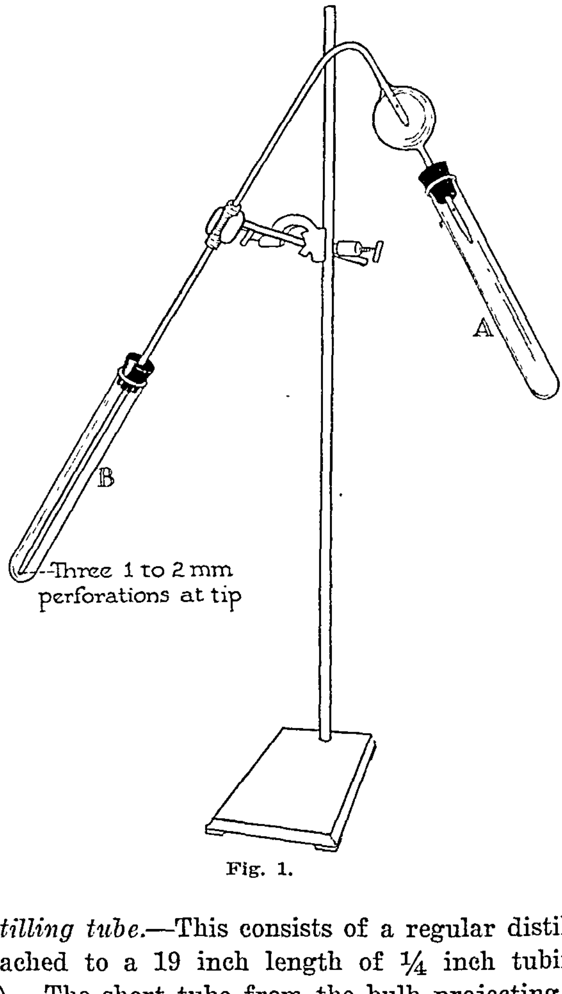

Fig. 1.

Improved distilling tube.—This consists of a regular distilling bulb of the Hopkins type attached to a 19 inch length of ¼ inch tubing at the angle indicated (Fig. 1). The short tube from the bulb projecting into the boiling tube *A* is made as short as possible, allowing space for a ⅛ inch hole in the

*From the Hull Laboratories of Physiological Chemistry of the University of Chicago. Received for publication, October, 1925.

side just below the rubber stopper. This hole ensures smooth boiling. At the very tip of the 19 inch length of ¼ inch tubing are three holes 1 to 2 mm. in diameter. These holes serve the double purpose of ensuring complete absorption of ammonia in the early stages of the distillation and of preventing the too rapid return of the distillate into the boiling tube *A* in case of changes in pressure during the distillation process. The usual micro-Kjeldahl or micro-

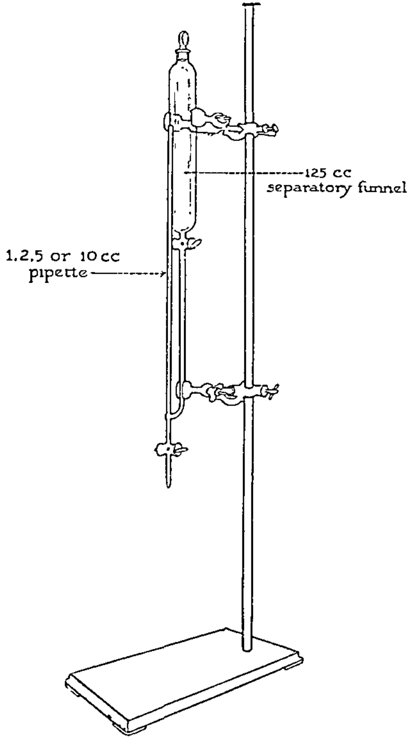

FIG. 2.

urea distillations are easily carried out in three to five minutes to the point where live steam issues through the distillate in *B*.

In the micro-Kjeldahl distillation the 1 c.c. of N/10 H_2SO_4 and 5 c.c. of ammonia-free water are measured into *B;* this is then securely attached to the mounted distilling tube. The latter (well protected by a cushion of heavy rubber tubing) is held in a clamp. To the contents of the digested mixture, consisting mainly of concentrated sulphuric acid, add 7 c.c. of distilled water and

a small knife point of 30 mesh zinc. Cool, and then add by means of a pipette 4 c.c. of the saturated NaOH solution in such a manner that the alkali flows down the side of the tube and forms the lower layer of strongly alkaline solution. Immediately after this addition connect the tube very securely with the distilling tube. Mix the contents of tube *A* thoroughly by shaking at tube *B*, using the clamp holding as a fulcrum. Then at once heat to boiling with a Bunsen burner, allowing the flame to play on the sides of the tube *A* just below the surface of the liquid rather than on the very bottom. Do not boil too vigorously but gently and continuously. When the contents begin to bump and crystallize, remove tube *B*, rinse off the tip of the distilling tube into *B* and then, by applying the flame on *A*, force out the small amount of water which has been drawn in at the tip during the washing. The material is then Nesslerized after appropriate dilution.

The sketch of the other apparatus (Fig. 2) is self-explanatory. Several precautions should be mentioned, however. The graduated pipettes used in assembling this apparatus should be high grade and carefully selected. The graduations on the 1 and 2 c.c. pipettes should be 1/100, and on the 5 and 10 c.c. 1/20 and 1/10 c.c., respectively. The length covered by the graduation should be 200 to 210 mm. for the 1 and 2 c.c. pipettes; 160 to 180 mm. for the 5 c.c. pipette, and 210 or more mm. for the 10 c.c. size. This apparatus we have found very valuable for measuring indicator solutions for P_H estimations and for measuring numerous reagents in microblood analysis. It has been found very satisfactory for measuring the cyanide solutions in uric acid estimations and for the titration of blood calcium by the permanganate method. In such very delicate titrations as the latter it is best to draw out the tip of the burette stopcock to a very fine point. One can easily obtain a tip of so small a bore that it delivers 100 to 110 drops per cubic centimeter. I have recently simplified this apparatus by substituting a three-way stopcock of the oblique bore type for the lower stopcock on the apparatus as illustrated. By so doing the stopcock on the separatory funnel part can be omitted.

A STABLE AND CONVENIENT UREASE REAGENT AND A MODIFIED BLOOD UREA METHOD*

By F. C. Koch, Ph.D., Chicago, Ill.

THE advantages of a stable solution of urease are, of course, obvious to all. In order to overcome the serious objection of instability so often encountered in the use of various convenient urease preparations, the well-known preservative action of glycerol toward many enzymes was applied to urease and found to fulfill the requirements.

The following formula has given in our hands very stable and satisfactory solutions of urease. Shake 15 grams of permutit with 200 c.c. of 2 per cent acetic acid. Decant the aqueous phase and wash two or three times with

*From the Hull Laboratories of Physiological Chemistry, University of Chicago.
Received for publication, October, 1925.

distilled water. To the permutit then add 50 c.c. of $N/1000$ H_2SO_4 and 30 grams of jack bean meal (Arlington Chemical Co.). Shake gently for one hour, then add 150 c.c. glycerol and mix very thoroughly. Filter through a creased filter. The filtration is slow and the product turbid. It may be necessary to change the filter paper after a few days in order to aid filtration. The filtrate is, however, very active and stable. The equivalent of 0.03 to 0.05 c.c. of such a glycerol extract of jack bean meal has been found to convert 2 mg. of urea nitrogen completely into ammonia in a 5 c.c. volume at 45° to 50° C. in fifteen minutes. Such extracts have been found to retain their full activity for as long as one year although stored at room temperature and exposed to the usual laboratory illumination. For ordinary blood urea estimations 1 c.c. of the 1:10 *freshly* diluted glycerol solution for 5 c.c. blood filtrate has been found reliable.

MODIFIED BLOOD UREA METHOD

The modified blood urea method can be employed only where sufficient blood is available to permit of preparing a separate blood filtrate for this specific purpose. The procedure is as follows:

Into a clean 1 × 8 inch pyrex test tube introduce accurately 2 c.c. of the well mixed blood. To this add 9 c.c. of distilled water, 2 drops of Folin's phosphate solution and 5 c.c. of the 1:10 *freshly* diluted glycerol urease solution. Mix well, stopper the tube with cotton and allow it to incubate for fifteen to thirty minutes at 50° C. Then add 2 c.c. of the 10 per cent sodium tungstate solution and complete the precipitation of the proteins by the usual addition of 2 c.c. of the 2/3 N sulphuric acid. Shake well, filter, and transfer 5 c.c. of the *clear* filtrate to a 1 × 8 inch pyrex test tube. Add the 2 c.c. of saturated borax solution, a small knife point of 30 mesh zinc, and distil with the tube described in the previous article.

This procedure enables one to carry out a smooth distillation without the usual serious foaming. We have found this modification very satisfactory for the routine laboratory classes, whereas by the older procedure we usually obtained very low results because of poor distillation.

AN IMPROVED EXTRACTION TUBE FOR CHOLESTEROL DETERMINATION*

By S. L. Leiboff, Ph.B., New York

IN July, 1925, I described in this Journal a special apparatus for the extraction of cholesterol from blood. It consisted of a small test tube constricted in the middle at the 5 c.c. mark. Above the constricted portion of the tube was placed a filter-paper disc containing the blood. The disc was perforated in the center by a small opening in order to allow the free passage of the chloroform.

*From the Biochemical Department of Lebanon Hospital Laboratory, New York
Received for publication, November 26, 1925.

a small knife point of 30 mesh zinc. Cool, and then add by means of a pipette 4 c.c. of the saturated NaOH solution in such a manner that the alkali flows down the side of the tube and forms the lower layer of strongly alkaline solution. Immediately after this addition connect the tube very securely with the distilling tube. Mix the contents of tube *A* thoroughly by shaking at tube *B*, using the clamp holding as a fulcrum. Then at once heat to boiling with a Bunsen burner, allowing the flame to play on the sides of the tube *A* just below the surface of the liquid rather than on the very bottom. Do not boil too vigorously but gently and continuously. When the contents begin to bump and crystallize, remove tube *B*, rinse off the tip of the distilling tube into *B* and then, by applying the flame on *A*, force out the small amount of water which has been drawn in at the tip during the washing. The material is then Nesslerized after appropriate dilution.

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The micrometer head, M, is metrically graduated over a distance of 25 mm. (maximum thrust) in divisions calibrated to 0.01 mm., and accurate to 0.0025 mm. by interpolation. The screw thread type of micrometer head has, instead of the usual flat face of the regular type, a pointed face, as appears in the photograph. It is essential that there be perfect contact between the micrometer head and the syringe plunger, which is insured by the accurately centered metal point. The accuracy is greater than that involved in polishing the head of the syringe plunger in a plane perpendicular to the axis of the plunger, or in drawing the syringe plunger to an accurately centered point. This micrometer head is set in a hole in a piece of inch brass rod reamed to fit. In order to insure rigidity, a thumb set screw, D, is inserted. On each side of

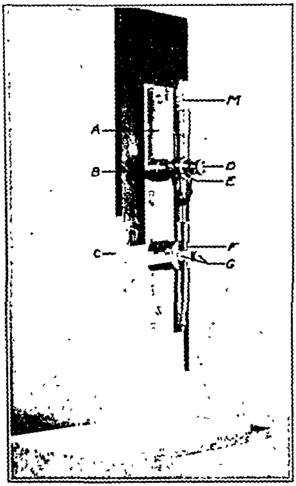

Fig. 1.

this micrometer holder are placed brass screws, E, set tight, which serve only to hold rubber bands which are attached to the syringe plunger, keeping firm contact between the plunger and the head. Contact of this sort pushes the plunger directly, with no turning.

The syringe itself is set on a brass rod, F, through which a hole to fit has been bored, the rod then being cut off through the center of the hole, leaving a semi-cylindrical groove for the syringe. To clamp the syringe in, a piece of heavy strip brass is bent to fit, and a piece of rubber tubing, cut in semi-cylindrical form is cemented in. This brace is fixed to the main stock by means of two knurled thumb screws, G, long enough so that the syringe may be loosened easily without removing the piece.

The vertical position of the micrometer head is insured by the large shoulder of the brass fixture, B, pressing against the flat metal strip facing,

A, used on the board. With the exception of the thumb screws, *D* and *E,* this piece, *B,* is made from one section of brass rod. From *B* this piece, 3/8 inch in diameter and threaded, extends through the board, being secured by a large thumb lock nut on the back of the board.

The syringe holder also extends through the board to be secured by a thumb lock nut in the rear, but the solid piece corresponding to the shoulder, *B,* of the micrometer holder is replaced by a similar thumb lock nut. The syringe may, therefore, be locked in a position centered under the point of the micrometer, being so made as to be set out or in from the board by means of the lock nuts, and movable sideways by a rotary motion. By loosening either of the set screws, the free rotary motion enables the swinging of the syringe out from under the micrometer for removal or refilling. Inasmuch as the useful stroke of the syringe plunger is greater than 25 mm., the maximum thrust of the micrometer, a syringe full of liquid may not be used without resetting the syringe. The syringe may be loosened and set higher. Perhaps it is preferable, however, to place a vertical slot instead of a hole through the plate *A* and through the board. The large thumb lock nuts enable one to fix the syringe holder very firmly, whereas the syringe position in the holder is best guaranteed by having the flange on the top of the barrel resting on the metal holder. Obviously the slightest slip of the barrel will make a measurement valueless, and such a slip might easily pass unnoticed.

The syringe may be either an ordinary 0.5 c.c. tuberculin syringe, or a 1.0 c.c. tuberculin syringe. Both fit equally well, since the difference is in the size of the plunger, and not in external dimensions. We have also another instrument built for a 2.0 c.c. Luer syringe, for use when greater amounts of liquid are to be measured. The best needles for the purpose are platinum-iridium needles, squared at the end. We have, however, been successful with ordinary steel needles, squared. A stroke of the syringe plunger of *m* millimeters ejects *n* c.c. of liquid with any one syringe, quite regardless of the size, shape, or condition of the needle, and a steel needle, carefully cared for has given us good results.

ACCURACY OF THE INSTRUMENT

Illustrating the accuracy of measurment of liquid, the figures for calibration of a 1.0 c.c. tuberculin syringe may be given. Glycerin was used for calibration, having a tested density of 1.248 at 21° C. Amounts ejected by turning 500 scale divisions on the micrometer (or 5 mm. in vertical push on the syringe) were weighed. Twelve weighings, covering the length of the syringe twice, gave as the weights in milligrams: 139.3, 138.9, 138.8, 138.8, 138.6, 139.0, 138.9, 138.9, 138.9, 138.8, 138.9, and 138.8. The average weight is 138.88 ± 0.047 mg., or 0.03 per cent error, as the mean average of the mean. The mean error of each determination is 0.164 mg., or 0.12 per cent error. On this basis one small scale division on the micrometer (0.01 mm. push) ejects from this syringe 0.0002226 c.c. This figure is of the nature of a factor; naturally, so small an amount would not be accurate to the number of points to which the figure is carried. Thus, if one wishes to measure 0.021 c.c., the syringe plunger must be pushed 94.3 one hundredths millimeters, or

that number of scale divisions. Five scale divisions eject only slightly over 0.001 c.c. It is thus apparent that the control over small amounts is good. With the 0.5 c.c. tuberculin syringe, our syringe gives for 0.021 c.c. 192.8 scale divisions, or approximately twice as many as needed with the 1.0 c.c. syringe. This smaller syringe is, therefore, capable of handling amounts one-half as great as the larger syringe with virtually the same accuracy.

That the liquid be flush with the tip of the needle at the start of measurement is essential; in our experience touching off squarely with the flat dry surface of a glass slide, or observing the surface with a hand lens, are satisfactory. In the latter method, a droplet formed at the tip of the needle may be drawn back by reversing the micrometer until the liquid is square with the opening by actual observation with a hand lens.

SUMMARY

A microsyringe is described, capable of measuring small amounts of liquid, from less than 0.001 c.c. to 1.0 c.c., with an error of not over 0.2 per cent, and, for appreciable amounts (e.g., 0.02 c.c.), less than 0.1 per cent.

The authors wish to acknowledge the careful work of Miss Esther Shultis in checking the calibration of the syringe, thereby virtually eliminating a personal factor in the calibrations.

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[1]Trevan: Lancet, April 22, 1922, i, 786.
[2]Bayne-Jones: Jour. of Immunol., November, 1924, ix, 481.

SOME USEFUL APPARATUS*

By Max Shaweker, M.D., Dover, Ohio

BEING situated at a considerable distance from a supply house, we are frequently called upon to rig up makeshifts of one sort or another. At times these makeshifts prove to be equal to the standard apparatus.

The first piece is a transfusion apparatus which has proved to be quite positive in action and almost a one man affair. It appears to be just the opposite of the latest types recommended in that it does not hurry the operation or move the blood rapidly. We have tried several of the valves recommended for directing the flow of blood from the donor to the recipient, but they have all proved to be unsatisfactory, either favoring clotting or not being air tight. They seem to require the blood to "squirt" through some valve or other when the natural motion of blood is one of smooth flowing. In our apparatus (Fig. 1) we have reamed out all the metal parts so as to provide a large bore and to favor flowing rather than "squirting." Then our syringe is the simple Bransford-Lewis type of 15 c.c. capacity which is operated by one hand leaving a free hand for adjustments and valving

*Read before the Fourth Annual Convention of the American Society of Clinical Pathologists, Philadelphia, May 20 to 23, 1925.

duty. The tube of this syringe can be paraffined if desired. The fingers used on the tubing, instead of clamps or valves, afford a sure working valve operation. The irrigation solution, either isotonic salt solution or a weak citrate solution, is placed in the upper jar and the conductor tube is connected to the large bore adapter (D) of the donor's needle or cannula, as the case may be. Another conductor tube is placed to the adapter (R) of the recipient's needle. This conductor tube is carried to a height just below the irrigating jar where a Y-tube (Y) is interposed so as to maintain a slight positive pressure in the tube system. These two tubes are kept parallel and are of the same caliber of tubing so as to be easily operated simultaneously with one clamp. Small caps (C) for the needle adapters are made by sealing

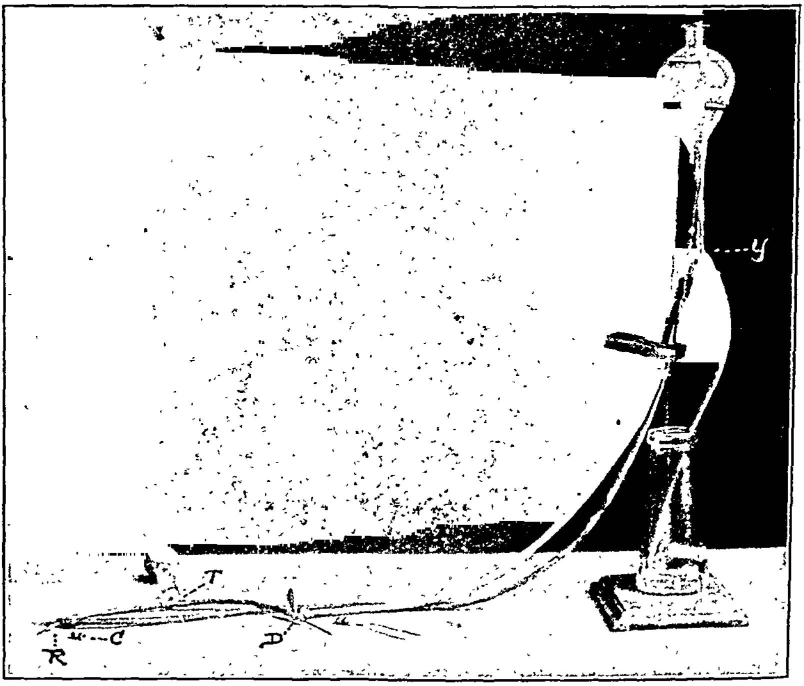

Fig. 1.

discarded Luer needle hubs with a drop of solder. By manipulating the clamp and the Bransford-Lewis syringe, the entire tube system is filled with solution and the air expelled. The veins are then entered using Lindeman cannula or some other large needle. The adapter caps are removed and the adapters are connected up to the needles. We usually use a Lindeman trocar needle for the donor and a platinum eighteen-gauge plain needle for the recipient. In transfusing no haste is required. The apparatus can be flushed frequently if desired, usually a short flush for each four or five syringe barrels of blood is sufficient. It can even be rested for a short time if filled with the citrate solution. Practically all the blood transfused is whole blood. A small portion of it may at times be citrated. In fact, I am not prepared to say whether or not whole blood has any advantages over properly citrated blood. At any rate, we make no effort to prevent a little citrate solution

getting into the recipient. This simple irrigating device can be applied to almost any whole blood apparatus by simply using needle adapters with side tubes. Very little chance exists for the recipient's blood entering the vein of the donor as the irrigating fluid enters just behind the donor's needle and flows toward the recipient. It may be that a slightly smaller syringe would work a little better. In fact, we have written to a manufacturer about this. We are now working on a valve to attach on the outside of the tubing which

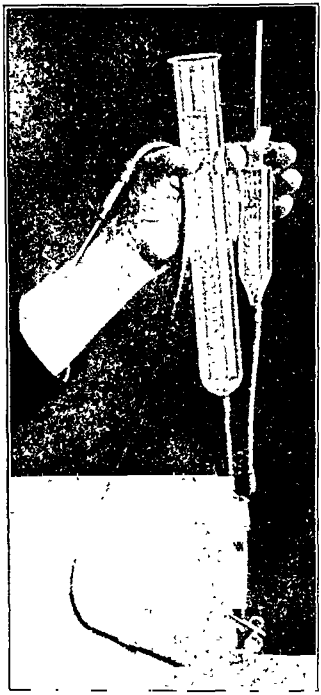

Fig. 2.

will span across the (T) tube, instead of using the fingers, but to date the fingers have the call.

Another apparatus which we use constantly and find very satisfactory is an infusion outfit for administering arsphenamine. This simple apparatus (Fig. 2) is made up of a 250 c.c. burette, the barrel of an Asepto syringe with a catheter tip, a rubber one-holed stopper with small piece of glass tubing, a Y-tube, and the conductor tubing. This will introduce first the isotonic salt solution, then the arsphenamine and follow with the salt solution again in a

semiautomatic manner which I will try to demonstrate. In this apparatus as in the transfusing apparatus we avoid stopcocks or valves in the tubing. The large burette is filled with salt solution which is allowed to expel all the air from the tubing and to back up into the small cylinder a short distance. The small cylinder is then clamped off and the arsphenamine, having been made up and alkalinized in the usual manner, is poured into the smaller cylinder after which the stopper with small glass tube is placed in the neck. The small glass tube is then made to just touch the arsphenamine solution. The clamp is now removed and the salt solution will back up to find a level in the small glass tube. Now the vein is entered and the solutions allowed to flow. The arsphenamine will not flow except for the very small amount represented in the small glass tube until the level of the lower end of the small tube is reached. After this the two tubes will fall equally, diluting the arsphenamine in the tubing as they flow. It has several advantages in that no stopcocks or other

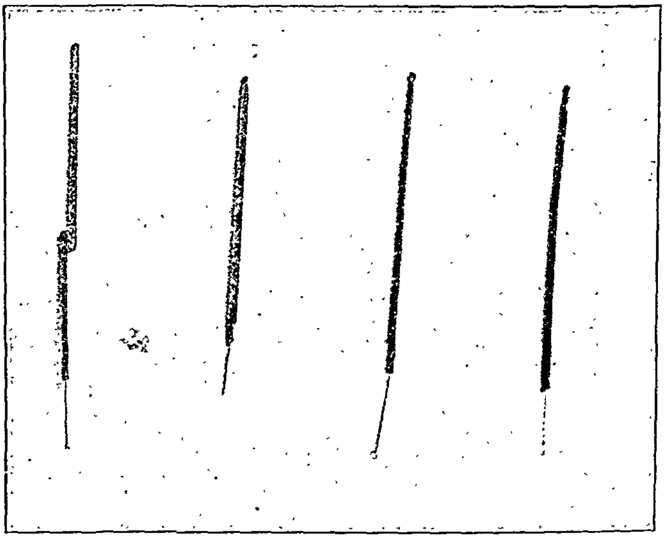

Fig. 3.

adjustments are required once it is started. The alkalinized arsphenamine can be kept quite cold and diluted only in distilled water until just before entering the vein. The salt solution can be brought well up to or slightly above body temperature, which will deliver at the needle practically an isotonic solution at body temperature.

Another small piece which has won a place in our laboratory is this style of handle for inoculating loops (Fig. 3), dissecting needles, etc. It is simply a short length of umbrella stay, the end of which is annealed by heating to a cherry red and the desired loop or needle clamped in with the blow of a small hammer. It has several advantages other than its cheapness in that it will not overheat with continued use, as will one which engaged the loop with a solid brass chuck or a solid glass rod. The loop cools rapidly and is sufficiently rigid, light and workable. By using a section near a joint a folding handle can be made which will carry well in a grip without continually bending off the needle,

URINARY PRESERVATIVES INCLUDING HEXAMETHYLENAMINE*

By Jeanette Allen Behre, Ph.D., and William Muhlberg, M.D.,
Cincinnati, Ohio

Introduction

The work reported in this paper was undertaken with the intent of finding a satisfactory preservative for samples of urine which are to be analyzed for clinical or other practical purposes. There seems to be no preservative in general use at the present time which can be considered ideal for this purpose. The requirements for such a preservative may be summarized as follows:

1. That it should preserve the urine from bacterial decomposition and the development of moulds or other growths for considerable periods of time under adverse conditions.
2. That it should not interfere either positively or negatively with any of the physical, chemical or microscopic tests in ordinary use.
3. That it be readily soluble.
4. That it should not interfere to any marked extent with the normal reaction of the urine.
5. That it should be a solid.
6. That its cost be reasonable.

Methods

With a view to finding a preservative which combined these conditions, we have investigated a number of varying substances. For the tests we used first normal urine, to which were added known amounts of glucose[1] and albumin.[2] The samples were kept in corked bottles in the incubator at 38° C. for periods of from four days to two weeks and were examined from time to time. These examinations included:

1. General macroscopic appearance of urine, odor, color, sediment, etc.
2. Sugar content as determined by the picric acid method of Benedict.[3]
3. Albumin by the sulphosalicylic acid method.[4]
4. Albumin by the nitric acid test.
5. Microscopic examination.
6. Reaction to litmus.

In further experiments the urine used was infected with samples already

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[1]J. T. Baker Chem. Co., Dextrose, C. P.

[2]Centrifuged sheep serum, diluted with 15 per cent NaCl.

[3]Benedict's Clinical Quantitative Test for Sugar in Urine, Hawk: *Practical Physiological Chemistry*, ed. 8, P. Blakiston's Son & Co., p. 665.

[4]Seven and one-half volumes of 3 per cent sulphosalicylic acid were added simultaneously to 2.5 volumes of urine and 2.5 volumes of each of a series of sheep serum standards corresponding to albumin concentrations of from 0.01 to 0.1 per cent.

containing bacteria, or with yeast. The preservatives which proved to be of most promise were also added to samples containing casts and red blood cells. The sodium nitroprusside test for acetone, and the Fehling test were also tried in connection with these.

PRESERVATIVES TRIED

The preservatives which we tried included the inorganic substances sodium fluoride, zinc chloride, boric acid and borax; and of organic substances, formaldehyde and toluene; certain phenols, such as thymol, tricresol, resorcinol and hexamethylresorcinol, benzoic and salicylic acids and their sodium salts, quinoline, camphor and menthol, acriflavine, chlorazene, and the so-called "Urofix" tablets.

The preservative which was decided upon as better than any of these, is a mixture of hexamethylenamine and salicylic acid. Before describing its advantages, however, a brief description of our results in connection with the other substances may be of interest.

Sodium fluoride is in most ways a very good preservative. It maintains the urine in a fresh condition for an almost unlimited period of time, in as low a concentration as 0.05 per cent and also does not interfere with sugar or albumin determinations at a concentration of 0.25 per cent. It produces a precipitate (probably calcium fluoride) in the urine, however, even in the lowest concentrations, which interferes with the microscopic examination and makes its use impracticable. Casts are apparently destroyed when sodium fluoride is added in any of the higher concentrations.

The combination of sodium fluoride with thymol, boric acid, sodium oxalate, sodium salicylate, sodium benzoate or zinc chloride did not improve the microscopic picture, nor could the sediment be washed free from the interfering precipitate with salt solutions, dilute acids or alkalis.

Potassium and ammonium fluoride yielded the same results.

Zinc chloride preserves the urine fairly well in amounts above 0.2 per cent and does not interfere with sugar reactions. It precipitates the albumin very appreciably, however, even when only 0.1 per cent is used, and it tends to disintegrate cells and form granular precipitates.

Boric acid does not interfere with the albumin reaction nor any of the microscopic constituents, but it does not preserve the urine under adverse conditions and interferes with the Benedict picric acid sugar determination in some positive way, giving sugar values which are too low. This is not due simply to the fact that the growth of yeast or sugar-destroying bacteria is not prevented, but it is a phenomenon which can be observed immediately after the boric acid is added, or in the presence of some efficient preservative, such as sodium fluoride or hexamethylenamine.

Borax has no recommendations, as it forms an alkaline urine, and even in connection with salicylic acid adds nothing as a preservative to the acid.

Formaldehyde is in many respects an ideal preservative. Concentrations of from 0.05 per cent to 0.1 per cent (depending upon the conditions and amount of infection) preserve the urine from bacteria and decomposition in

most cases without changing the microscopic picture or the albumin figures and without appreciably affecting the sugar value.

There are, however, some disadvantages to the use of formaldehyde as a preservative. In the first place, it is a liquid, which is the most important reason for discarding it. In larger amounts, it interferes with the sugar determinations which depend upon reduction. With the Benedict picric acid method it is found that urines containing 0.05 per cent of formaldehyde (1 drop "formalin" to 40 c.c. urine) show slightly more color than the urine without preservative and that this color increases with perfect gradation as the formaldehyde is increased. One-tenth per cent of formaldehyde in urines which are apparently sugar-free gives a color corresponding to about 0.1 per cent of sugar. With 0.5 per cent formaldehyde (about 10 drops of "formalin" per 40 c.c. urine) the color is very much deeper than the color corresponding to 0.1 per cent of sugar. The increase in color due to the smaller amounts of formaldehyde which would ordinarily be used for preserving the urine would not, however, be sufficient to interfere with the clinical determination of the sugar. In larger amounts, also (10 drops "formalin" to 40 c.c. urine) formaldehyde produces a sediment of globules or crystalline rosettes which interferes with the microscopic picture. Often complete disintegration of the cellular material takes place in the urine when these higher concentrations of formaldehyde are used.

Toluene is a fairly good preservative, but it is not as effective as formaldehyde, and is also a liquid.

Thymol was found to be an almost ideal preservative within certain rather restricted limits of concentration. These suitable concentrations are very low, from approximately 0.025 per cent to 0.1 per cent. When thymol is used in a percentage between these limits, the urine is beautifully preserved from bacterial or yeast invasions; no interference is found with the sugar determinations or with the albumin, as determined by the sulphosalicylic acid method, and the microscopic picture is found to be maintained in unusually good condition.

Larger amounts of thymol, however, form globular, gelatinous or chalky sediments. Also, even a very minute amount of thymol forms a small very compact ring with nitric acid, which in the presence of albumin is distinguishable from the albumin ring, lying below it and having a sharper appearance, but which might be mistaken for albumin in the latter's absence. Thymol has also been reported to interfere with the iodometric methods for acetone determination.

Even with these disadvantages, thymol was probably on the whole the second best preservative which we found.

Tricresol, Resorcinol and Hexamethylresorcinol are out of the question, since they all react in the picric acid sugar method, giving dark colors, and make turbid or milky or very dark colored urines with spongy or oily sediments.

Salicylic acid forms a moderately good preservative in amounts less than 0.1 per cent as far as sugar and microscopic examinations are concerned, but even at a concentration of 0.05 per cent it precipitates the albumin markedly, while in larger amounts it tends to increase the apparent sugar content and

to make the urine dark with a fine, dark sediment. It also gives the urine a strong acid reaction.

Sodium salicylate used in amounts which do not increase the specific gravity excessively does not preserve the urine adequately from bacteria or prevent glycolysis.

Sodium benzoate is open to the same objection.

Quinoline used in small amounts did not interfere with sugar or albumin, but it did not preserve the urine as adequately as many other preservatives, and its very peculiar and penetrating odor makes it highly undesirable for this use.

Camphor and menthol were not found to be adequate preservatives in moderate amounts and were discarded almost immediately.

Acriflavine (neutral acriflavine—Abbott) proved to be a fairly good urinary preservative even in such small amounts as 0.01 per cent, but the urine was so intensely colored by the dye that its use was out of the question.

Chlorazene (sodium-para-toluene-sulphochloramide—Dakin's antiseptic) was more promising. Used in concentrations of from 0.3 per cent to 0.75 per cent it preserved the sugar and albumin well, and did not interfere with the microscopic picture. The urine was kept clear and light, with very slight sediment. Smaller amounts, however, did not preserve the urine, while larger amounts bleached it to a chalky or completely colorless condition with no sediment. Casts were usually injured, even when the smaller concentrations of chlorazene were used.

The urofix tablets, put out by Wolf, were given a careful consideration, especially in connection with the hexamethylenamine-salicylic acid mixture which we chose as being the best preservative. It was found that one urofix tablet (supposed to be adequate for the preservation of 2 oz.) would preserve 40 c.c. of urine moderately well under favorable conditions, but that it often failed to keep the urine in good condition when bacteria were introduced. Even under the best conditions one tablet produced a brownish sediment, usually including small dark granules, which appeared in the microscopic picture. Two urofix tablets, which preserved the urine from infection in most cases, produced much more abundant sediments, which often interfered with the microscopic picture. A noticeable amount of albumin was also often precipitated by the urofix tablets.

HEXAMETHYLENAMINE AND SALICYLIC ACID

The preservative which was found to be the best, almost without any question, is a combination of hexamethylenetetramine (urotropin) with salicylic acid. As is well known, hexamethylenamine liberates formaldehyde and ammonia in acid solution, and it seemed that theoretically this would make an ideal preservative which would have all of the advantages of formaldehyde with the added advantage of being in a solid form. This expectation appeared to be fully justified. In many cases the new preservative seemed to maintain the normal condition of the urine more satisfactorily than did formaldehyde. The concentrations which were chosen as being the best were 0.3 per cent hexamethylenamine and 0.2 per cent salicylic acid.

Hexamethylenamine alone was found to preserve acid urines moderately well, but, with the salicylic acid added, even alkaline urines and urines badly infected with bacteria are remarkably well preserved. Salicylic acid was chosen as being a good preservative in itself. Benzoic acid did not give quite such good results. The disadvantages found in connection with using salicylic acid alone were all obviated when it was combined with the base. The reaction of the urine with the preservative added is amphoteric to litmus, sometimes more acid, sometimes more alkaline, but always at least slightly amphoteric. The amount of salicylic acid which we decided to use is such that even urines made distinctly alkaline with sodium carbonate turned blue litmus red when the preservative had dissolved in them, and even these urines were well preserved.

With the new preservative sugar and albumin concentrations are maintained, and there is no appreciable interference with any of the tests, including Fehling's and the nitroprusside and iodoform tests for acetone. Casts and red cells are preserved. Yeast does not form gas with the preservative alone in sugar-free urine, and fermentation of sugar in the urine is repressed though not entirely inhibited in the presence of the preservative. Two sets of normal urine were kept with this preservative for twelve weeks without showing any change in sugar or albumin content and giving an almost perfect microscopic picture at the end of the time, with epithelial cells intact and no bacteria. Many sets were kept in excellent condition for at least six weeks.[5]

That formaldehyde really is formed in the urine from this combination is unquestionable. Water solutions of the mixture in the same concentration give a faint though characteristic odor of formaldehyde after a few days, and urines containing it have the same sweetish almost unmistakable odor as do urines which are preserved with very small amounts of formaldehyde. With larger amounts of the mixture, the odor becomes more characteristically that of formaldehyde.

Furthermore, when the preservative is added to sugar-free urine, and the picric acid test performed at intervals, it is found that a very slight increase in the faint color given by the urine alone occurs from day to day. When twice the amount of preservative is used, this color, after twenty-four hours, matches the color given by 0.05 per cent of formaldehyde in the same urine. This increase in color, like that given by formaldehyde itself, is noticeable only in urines of which the sugar content is very low, and it is not great enough to make any appreciable difference in the results of the sugar determination. The formaldehyde is evidently produced gradually as the reaction goes on.

It is interesting to note also that salicylic acid in concentrations exceeding its solubility in pure water is dissolved at once when sufficient hexamethylenamine is added, and that when less hexamethylenamine is added the solid salicylic acid, which is not at first dissolved in the presence of the base, gradually does go into solution, evidently being used up in the course of the reaction.

It seems probable that the salicylic acid acts as a preservative of the urine during the first period while the formaldehyde is being produced.

It would be of considerable advantage for routine work if the preservative

[5]Since the completion of this investigation the new preservative has been in use for four months in the Union Central Life Insurance Company with entirely satisfactory results.

mixture could be put into tablet form. We have not yet found an entirely satisfactory way of doing this, though we hope to do so eventually. It has been found perfectly satisfactory, however, even when routine work is being done on a large scale, to use an intimate mixture containing three parts of powdered hexamethylenamine and two parts of salicylic acid. This is added to the urine in the proportion of 50 mg. to 10 c.c. of urine.

SUMMARY

A brief description is given of the advantages and disadvantages found in connection with a number of substances which were tried and discarded as urinary preservatives.

A preservative which seems to fulfill the requirements for practical urinary analysis is described. This consists of a mixture of hexamethylenetetramine and salicylic acid in the ratio of three parts to two. Fifty mg. of the mixture are used for the preservation of every 10 c.c. of urine.

A VALVELESS ADAPTER FOR A TWO-WAY SYRINGE*

BY PAUL G. WESTON, M.D., JAMESTOWN, N. Y.

FOR the purpose of dispensing with valves in a two-way syringe, an adapter using the burette stopcock principle was made. The projections *A A* are adapters for a Luer socket and *B*, for a Luer syringe nozzle. *C* is a small screw holding the two parts together.

For use the piston is withdrawn and the syringe barrel fills through the adapter on the right. The syringe is then given a half turn and its contents

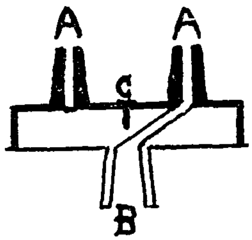

Fig. 1.

ejected through the adapter on the left. The process is repeated until the required amount of fluid is injected or, in the case of aspiration, until the desired amount is withdrawn. A small stud, not shown in the diagram, prevents more than a half turn being made.

*Received for publication, December 7, 1925.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

Snell, A. M., Greene, C. H., and Rowntree, L. G.: Diseases of the Liver. II. A Comparative Study of Certain Tests for Hepatic Function in Experimental Obstructive Jaundice. Arch. Int. Med., August, 1925, xxxvi, 273.

In the fructose tolerance test given to dogs there was a definite reduction in tolerance, a rise of 40 mg. per cent above the normal being the rule.

There was a definite fall in the nonprotein nitrogen following the development of jaundice, the decrease involving both the relative and absolute urea values The values, however, as a rule, were within the lower limits of normal.

The changes in the bilirubin content of the serum are characteristic and constitute a test of definite value. The findings closely parallel those of the tetrachlor test. Dye retention first appears with the development of bilirubinemia.

The coagulation time is prolonged though the fibrin content was increased and serum calcium unchanged.

Greene, C. H., McVickar, C. S., Rowntree, L. G., and Walters, W.: Diseases of the Liver. III. A Comparative Study of Certain Tests for Hepatic Function in Patients with Obstructive Jaundice. Arch. Int. Med., September, 1925, xxxvi, 418.

The following tests were studied: fructose tolerance, blood-sugar level; nitrogen partition; bile pigments in the blood; and phenoltetrachlorphthalein test.

The greater number of tests studied failed to show changes sufficiently significant to be of clinical value. The most valuable clinically were the studies of the serum bilirubin content, in which an increase occurs before there is demonstrable jaundice, and of the tetrachlor test, which closely parallels the pigment curve.

Greene, C. H., McVickar, C. S., Walters, W., and Rowntree, L. G.: Diseases of the Liver. IV. Functional Tests in Cases of Carcinoma of the Liver and Biliary Tract. Arch. Int. Med., October, 1925, xxxvi, 542.

Report of various functional tests in malignancy, the cases being grouped as follows:

I. Metastatic carcinoma of the liver associated with jaundice, 14.

II. Metastatic carcinoma of the liver without jaundice, 18.

III. Intraabdominal carcinoma without demonstrable involvement of the liver, 17.

In eight cases in Group I fructose tolerance was lowered; this also occurred in one case in Group II and in two cases in Group III. Because of the irregularity in the results and the frequent association of conditions affecting the carbohydrate mechanism, the authors regard this test as of little clinical value.

There were no significant changes in the blood-sugar level, nor were any changes found in the nonprotein nitrogen of the blood which could be attributed to the carcinomatous process. The most useful clinical tests were the determination of the serum bilirubin and the tetrachlorphthalein excretion.

In Group I the icterus index was increased, and a direct van den Bergh reaction was obtained.

In Group II a zone of latent jaundice was obtained in three cases, normal values being found in Group III.

The findings were comparable to those already reported and of definite clinical value.

Tetrachlorphthalein readings:

In Group I the maximal reading in one hour was 51 per cent, the average being 21 per cent.

In Group II the maximal reading was 28 per cent, definite retention occurring in eleven of the eighteen cases. A normal reading was noted in five of these cases.

In Group III three cases showed slight retention, two questionable retention, and the others were normal.

The authors believe that the serum bilirubin and tetrachlor tests are the only tests of those studied which are of cinical value in the early diagnosis of carcinoma of the liver and biliary tract.

Larson, W. P., and Halvorson, W. O.: The Effect of Concentration upon the Neutralization of Toxin by Sodium Ricinoleate. Proc. Soc. Exper. Biol. and Med., 1924, xxi, 278.

Continuing their studies of the action of surface depressants on bacterial toxins, the authors present the results of an attempt to show the effect of concentration of both soap and toxin upon the efficiency of detoxification.

Their experiments indicate that concentrations of soap below 5 per cent do not completely detoxify and that equilibrium is reached within a comparatively short time.

Other experiments show that the action of soap on toxin is a reversible one, that the toxin is not destroyed, and that the neutralization of toxin by sodium ricinoleate is an adsorption phenomenon.

Larson, W. P., and Colby, W.: Immunization Against Scarlet Fever Using Sodium Ricinoleate as a Detoxifying Agent. Proc. Exper. Biol. and Med., 1925, xxii, 549.

Scarlet fever toxin, the potency of which was 15,000 skin test doses per c.c., was mixed with a sodium ricinoleate solution in amounts sufficient to make a final soap concentration of 1 per cent, and this mixture was used to immunize human beings.

As the toxin had been completely neutralized it was found that 1000 skin test doses could be given to children with entire safety. Using such a "neutralized" toxin, 148 children from one to sixteen years of age who gave positive Dick tests, were injected with varying amounts of the soap-toxin mixture and, after one such injection, Dick retested in five to twenty-one days.

Three weeks after this single injection, 96 per cent were Dick negative. In 15 per cent there was a slight local reaction appearing within twelve hours and gone within thirty-six hours.

A group of adults were given 2000 skin test doses of the soap-toxin mixture without any reaction, 37.5 per cent being Dick negative in seventeen days. Larger doses, 4000 units, are recommended for adults. The soap-toxin mixture must be perfectly clear, should stand at room temperature for four hours to allow an equilibrium to become established, and should be kept in hard glass containers.

Larson, W. P., Howard, E. W., and Eder, H.: Antidiphtheretic Immunization Using Sodium Ricinoleate as a Detoxifying Agent. Proc. Soc. Exper. Biol. and Med., 1925, xxii, 552.

The ability of sodium ricinoleate to detoxify bacterial toxins has been pointed out in previous communications by Larson and his associates, and the present paper reports the results of studies conducted to determine if this property could be utilized in the active immunization of children and adults against diphtheria, using such a detoxified toxin.

A quantity of diphtheria toxin was mixed with a 1 per cent solution of sodium ricinoleate so that 1 c.c. of the mixture contained 0.125 L + toxin, and 1 c.c. of this mixture was injected.

Neither local nor general reactions occurred in 149 children and adults so treated. Of those so injected, 69 were retested within six weeks, 50.2 per cent giving a negative Schick reaction.

The method is reported as feasible for practical use and very effective, a majority of those so treated giving a negative Schick reaction within four weeks after injection of one dose.

Larson, W. P., Halvorson, H. O., Evans, W. D. and Green, R. G.: The Effect of Surface Tension Depressants Upon Bacterial Toxins. Colloid Symposium Monograph, New York, J. J. Little and Ives Co.

The authors report the effects of sodium soaps of various fatty acids on bacterial growth and bacterial toxins.

Soaps which form colloidal solutions do not appear to adsorb upon bacteria or their toxins.

Sodium ricinoleate, which seems to form a true solution, has a marked effect upon bacterial toxins in that, without any change in their antigenic effects, the toxins no longer produce toxic effects when injected into animals. This surface depressant action completely destroys the toxic action of tetanic, diphtheritic, and scarlatinal toxins so that several hundred fatal doses may be injected without injurious effect.

Injection of such detoxified toxins is followed by the production of immune bodies against the normal toxins and such large doses may be used that immunization occurs rapidly.

On the basis of these results soap-detoxified toxins have been used in the immunization of human beings against diphtheric and scarlatinal streptococcic toxins.

It is imperative that the soap must form a clear solution, that no foreign particles be present, and that the solutions remain perfectly clear. It has also been found necessary to allow the mixture to stand for some time (four hours at room temperature) to permit an equilibrium to be established.

The action of the soap appears to be an adsorption phenomenon. The concentration of both soap and toxin is of importance.

Experiments are cited which indicate that a toxic mixture may be made nontoxic by diluting the toxin and keeping the concentration of soap constant, or by increasing the soap concentration without changing the concentration of the toxin.

The assumption is that in free toxin preparations the toxin exists in colloidal aggregates capable of dispersion and that to secure complete protection the aggregates must be dispersed and a layer of soap adsorbed over the entire surface of the toxin molecule.

Dispersion is brought about either by adding a sufficiently concentrated soap solution, or by diluting the toxin.

Concentration of the toxin, rather than the dosage, appears to be the prime factor in controlling the amount of antibody formed.

Black, J. H., and Moore, M. C.: Pollen Therapy with Protein-free Extracts. Jour. Am. Med. Assn., January 30, 1926, lxxxvi, 5:324.

Evidence is presented that the activity of pollen extracts is unimpaired by the removal of the protein constituents and that standardization by nitrogen content of complement fixation does not indicate the content of active substance.

Scudder, S. A: A Comparative Study of the Value of Stained Smears and Cultures in the Diagnosis of Gonorrheal Vulvovaginitis. Jour. Urol., November, 1925, xiv 5, 429.

In a carefully controlled study of twenty-four cases, several modifications of Gram's stain were used, the most satisfactory being the akaline method described by Burke, using 95 per cent alcohol as a decolorizer. The exact details of the technic used by the author are not given in the original paper.

Cultures were made on a modification of the Thalmann medium (veal-infusion-glycerol agar to which urine and Grubler's Iodgrun were added) prepared as described by Torrey (Jour. Infect. Dis., 1922, xxxi, 125).

Semisolid agar to which a few drops (less than 0.5 c.c.) of ascitic fluid, or a good grade of horse plasma without preservative, were added was found to be an excellent emergency medium.

Organisms culturally and morphologically similar but not gonococci were not encountered.

The fermentation test is the most reliable means of differentiation.

The stained smear, when carefully made, checked and controlled, is a reliable method of diagnosis.

The paper contains much informative data, but the methods used are only indefinitely described, reference to the original papers mentioned being necessary to ascertain the exact technic.

Reith, A. F.: Bacteria in Muscular Tissues and Blood of Apparently Normal Animals, A Preliminary Report. Jour. Am. Med. Assn., January 30, 1926, lxxxvi, 5, 325.

Cultures of the muscular tissues of slaughtered dogs show the presence of bacteria in 77 per cent of 216 samples; 57 per cent contained anaerobes.

In healthy, live hogs, rabbits, and guinea pigs 83 per cent of 108 samples contained bacteria, anaerobes being found in 49 per cent.

Blood cultures from healthy animals were positive in 67 per cent of 18 samples, anaerobes being found in 44 per cent.

The investigations were part of a study of meat spoilage.

Jackson, G.: Crystal Violet and Erythrosin in Plant Anatomy. Stain Technology, January, 1926, xii, 1:33.

Satisfactory differentiation between liquefied and nonliquefied cells is obtained by the following technic:

1. Remove paraffin with xylol.
2. Remove xylol with absolute alcohol.
3. Ninety-five per cent alcohol.
4. Crystal Violet, 1 per cent in distilled water, fifteen minutes.
5. Rinse quickly in water.
6. Dehydrate quickly but thoroughly in 95 per cent and absolute alcohol.
7. Erythrosin, saturated solution in clove oil, one to five minutes.
8. Absolute alcohol and xylol, equal parts, one to two minutes.
9. Xylol.
10. Mount in balsam.

Genung, Eliz. F.: Basic Fuchsin as an Indicator in Endo's Medium. Stain Technology, January, 1926, i, No. 1, 41.

A method is described for preparing uniform solutions for Endo's medium.

The stock solution of fuchsin is made by dissolving 10 grams in 100 c.c. of 95 per cent alcohol. Dilutions are made in test tubes by adding to 1 c.c. of the stock solution, 1 c.c. (1:1), 2 c.c. (1:2), 3 c.c. (1:3), 4 c.c. (1:4), and 5 c.c. (1:5) of 95 per cent alcohol. To 10 c.c. of 2.5 per cent sodium sulphite solution is added 0.5 c.c. of each of the above dilutions. The tubes are allowed to stand and the lowest dilution of fuchsin, which is decolorized promptly, is used in the preparation of Endo's medium. The reaction of the agar is best placed at PH 7.4.

When a new lot of dye is used it should be tested with colon, aerogenes, and typhoid bacilli.

Harned, B. K.: The Sugar Content of Blood. Jour. Biol. Chem., October, 1925, xlv, 3, 555.

The Folin-Wu blood-sugar technic applied to blood filtrates after mercuric nitrate precipitation gives a filtrate relatively free from nonsugar interfering components; the results are from 17 to 25 per cent lower than those obtained with the Folin-Wu method and are in accord with Benedict's latest method.

Method.—Mercuric nitrate reagent: 348 gm. of mercuric nitrate are shaken in a liter flask with 900 c.c. of water for ten to fifteen minutes. Concentrated nitric acid is then added, a few c.c. at a time, and later drop by drop until the mercuric nitrate is dissolved. The solution is then made up to one liter.

1. In an Erlenmeyer flask dilute 1 volume of blood with 8 volumes of water.
2. While rotating the mixture add 1 volume of mercuric nitrate mixture, a little at a time. The proteins are precipitated in a jelly-like mass.
3. Stopper the flask and shake vigorously to break up the precipitate.

4. Filter.

5. Add solid sodium bicarbonate to the filtrate until effervescence ceases and the reaction is faintly alkaline to litmus.

6. Remove the resultant precipitate by filtration through a double filter.

7. Add dried and finely powdered potassium bisulphate until a drop of the mixture gives with tropeolin 00 on a spot plate the intermediate color shade.

8. Remove traces of mercury from the solution by precipitation with hydrogen sulphide which is first passed through water.

9. After filtering off the mercuric sulphide, blow excess hydrogen sulphide out of the solution by a current of air which first passes through a wash bottle.

The solution is then carried through the usual Folin-Wu technic, the standard being brought to the same reaction as the unknown by the addition of potassium bisulphate.

The method is not advocated as a routine procedure but is recommended as a technic which can be relied upon for the determination of the absolute blood-sugar level.

Holmes, W. C.: The Oxidation Products of Methylene Blue. Stain Technology, January, 1926, i, 1, 17.

The preparation of a new product, Azure C, which has valuable properties as a nuclear and bacterial stain in tissue and which may be substituted for Azure A in the MacNeal tetrachrome blood stain is described.

Dissolve 2.5 grams of methylene blue in 200 c.c. of water and bring to a boil. While boiling add a hot solution containing 5 c.c. of concentrated HCl (36 per cent) in 100 c.c. of water and 2.5 grams of potassium bichromate. Place in the autoclave for one hour at 115 to 120° C.

The resultant product has been termed Azure C. As a tissue and bacterial stain it is used as follows:

1. Xylol—3 min.
2. Absolute alcohol—3 min.
3. Ninety-five per cent alcohol—3 min.
4. Water—3 min.
5. Stain in the following for 5 minutes:

Azure C	1.5 gm.
Distilled water	100.0 c.c.

Note.—As a PH of 7.5 or greater is necessary for consistent results, 1 per cent of disodium hydrogen phosphate has been added to the azure. This has been found to be an essential, as in commercial production it is found that there often remains in the dry dye quite a little residual acid which must be neutralized. The presence of this phosphate does not interfere with the utility of the azure in other combinations.

6. Absolute methyl alcohol, five to ten seconds.

Note.—This is an important step preparatory to differentiation. The sections should be left in the methyl alcohol until no more free blue is apparently washed out; this is ordinarily accomplished in a few seconds. In case the final result shows a lack of sharpness of nuclear and bacterial staining, it is probably due to improper treatment in this step.

7. Differentiate and counterstain in:

Eosin Y and Orange II mixture	0.050 gm.
Glacial acetic acid	1.0 c.c.
Absolute ethyl alcohol	99.0 c.c.

Note.—Carry this step until no more free blue is washed out of the section, generally thirty to forty seconds. With steps 5 and 6 properly accomplished, it is difficult to over-differentiate, and little fear need be entertained in that direction. The eosin Y and orange II mixture referred to, is a mixture of eosin Y (C.I.768) and orange II (acid orange Y, C.I.151).

8. Absolute alcohol, 1 to 2 min.
9. Absolute alcohol, 1 to 2 min.
10. Xylol, 1 to 2 min.
11. Xylol, 1 to 2 min.
12. Xylol Balsam.

In practice the writers employ Coplin jars in this staining technic, using the staining solution and alcohols for a considerable period of time. There seems to be little deterioration in the solutions, some apparent discoloration not interfering with consistent results.

The stain may be used after formalin or Kaiserling fixation.

Ackman, F. D.: **The Relation Between Gastric Acidity and the Hydrogen Concentration of the Urine with a Study of the Effect of Histamine.** Canadian Med. Assn. Jour., 1925, xv, 1099.

The method of Henderson and Palmer (Jour. Biol. Chem., 1912, xiii, 393) was used for the determination of the urinary PH, except that 2 per cent aqueous alizarin was used where the PH was less than 7.4, and 1 per cent alcoholic phenolphthalein for PH of 8.0 or over.

The fractional method of gastric analysis was used in this investigation.

Studies were made of 67 normal subjects, 31 cases of achlorhydria from various causes, and 15 cases of hyperchlorhydria.

Conclusions:

1. The occurrence of the "alkaline tide" is corroborated, the lowest urinary acidity occurring usually three hours after a meal.
2. A relatively high acidity occurring at 9 A.M., 1 P.M. and 5 P.M. was not explained.
3. In cases of normal gastric acidity, the degree of urinary acidity varies inversely with the gastric acidity.
4. In hyperchlorhydria the daily urine tends slowly toward alkalinity; in achlorhydria the alkaline tide is wanting.
5. A high carbohydrate diet produces little or no alkaline tide; a high protein diet produces a conspicuous tide, due to differences in the gastric stimulation.
6. In starvation the alkaline tide is eliminated.
7. A meal always produces a change in the urinary reaction in normal individuals.
8. The evidence supports the gastric origin of the alkaline tide, and, when gastric analysis is impossible, the hourly PH may furnish information of clinical value concerning the secretory ability of the stomach.
9. Histamine, subcutaneously, stimulates the gastric secretion.

Hogue, M. J.: **Staining Protozoa with Janus Green B.** Stain Technology, January, 1926, i, 1:35.

One drop of 0.5 per cent aqueous solution to 10 to 20 drops normal saline (for parasitic) or water (for free living protozoa) added directly to a drop of culture will give a satisfactory flagella stain.

A dilution of 1:20 of 0.5 aqueous solution of the dye, will give vital staining, the protozoa finally becoming a deep pink as they dye.

Stadie, W. C., and Ross, E. C.: **A Micro Method for the Determination of Base in Blood and Serum and Other Biological Materials.** Jour. Biol. Chem., October, 1925, xlv, 3:735.

A method eliminating various sources of error.

Materials needed:

Standardized N/50 NaOH.

Benzidin hydrochloride solution.

Benzidin	4 gm.
N HCl	45 c.c.

Dissolve and make up to 250 c.c. Filter if necessary. This solution is standardized by titrating 2 c.c. against N/50 NaOH, using phenol red. It keeps indefinitely, requiring occasional filtration. It is best to restandardize about once a week, although as a rule the titer is unchanged over two to four weeks. Its equivalent concentration is about:

Benzidin hydrochloride	175 m.-Eq. per liter.
Excess HCl	5 " " "
Total	180 " " "

10 c.c. burettes graduated in 0.05 c.c.

50 c.c. silica beakers.

METHOD

Place 1 c.c. of serum or an aliquot of a trichloracetic acid filtrate of whole blood or cells (see below) corresponding to about 1 c.c. of blood in a 50 c.c. silica beaker. Add 0.5 c.c. of concentrated sulphuric acid and evaporate at low heat (about 90° C.) on a sand bath until practically all the water is driven off. Add 1 c.c. of concentrated nitric acid and continue heating, raising the flame from time to time. Add 1 c.c. of nitric acid occasionally to aid in the oxidation of organic matter. When the full heat of a triple burner has been reached and a clear white ash obtained, transfer to a triangle and heat fifteen minutes at bright red heat, allowing the flame to play over the entire outside of the beaker. This as a rule suffices to drive off all excess of sulphuric acid. Let the beaker cool to room temperature; add 15 c.c. of water. If preferred, the ash may be dissolved and quantitatively transferred to a 20 c.c. flask for precipitation. The ash readily dissolves with a little stirring. To be certain that no excess of sulphuric acid is present, add a drop of phenol red and titrate with N/50 NaOH. Rarely is more than 0.05 c.c. required. This amount may be neglected, but if more is necessary a correction must be made for it in the calculation. Add 2 c.c. of standardized benzidin hydrochloride and filter through a small dry quantitative filter into a dry Erlenmeyer flask. Titrate 15 c.c. of the filtrate with N/50 NaOH, adding more indicator if necessary.

Calculation of Milli-Equivalents of Base per Liter of Blood or Serum. For 1 c.c. of blood or serum analyzed as above we have,

m.-Eq. base per liter = (titer of 2 c.c. of benzidin HCl- $\frac{17}{15}$ titer of 15 c.c. of filtrate) = c.c. N/50 NaOH × 0.02 × 1000.

If the amounts given above are varied as occasion may direct, we have for N/50 NaOH.

m.-Eq. of base per liter or per kilo = 0.02 (titer of x. c.c. benzidin HCl $-\frac{\text{total volume}}{\text{aliquot}}$ × titer aliquot) ÷ amount of material in liters or kilos.

If more than 0.05 c.c. of N/50 alkali are required for the end-point of the ignited ash subtract from the titer of 2 c.c. of benzidin HCl in above formula. Allowance, of course, is made in the "total volume" for the added volume which is increased by the added alkali.

Calculation of Base Bound to Phosphate. As a rule the base in blood bound as phosphate is less than two milli-equivalents per liter and may for most purposes be neglected. Since the base as phosphate is not determined in this method, it must be separately determined when the phosphate concentration is a significant part of the total. The micromethods of Tisdall (1922) and Briggs (1924) are available. To the base as determined above add one milli-equivalent for each milli-equivalent of phosphorus. In the ignited ash, the phosphate is present as metaphosphate BPO_3 binding one equivalent of base per mol of phosphorus. If desired the phosphates may be removed by the method described below and the total base (including phosphate base) determined.

Preparation of Trichloracetic Acid Filtrate of Whole Blood or Red Cells. Place 3 c.c. of whole blood (or about 3 gm. accurately weighed of red cells) in a 50 c.c. volumetric flask. Add about 20 c.c. of water, and when hemolysis is complete add slowly, with vigorous shaking, 25 c.c. of a ten per cent trichloracetic acid solution. Make up to volume and filter. Yield about 45 c.c. Twenty c.c. of the filtrate are ashed as above.

When the ratio in equivalents of phosphate to base exceeds 10-150, the removal of phosphates becomes necessary and is achieved by the following method:

Technic for the Removal of Phosphate. The material (urine, tissue, gastric contents, feces, etc.) in an amount sufficient to give about 0.100 to 0.200 milli-equivalent of base is digested in a silica beaker with sulphuric and nitric acids until the organic matter is oxidized. Do not ignite to complete dryness at any time. Transfer to a 25 c.c. volumetric flask, using about 15 c.c. of H_2O; add a few drops of phenol red; neutralize with 4 N NH_4OH (1:4 concentrated NH_4OH). Render just acid with a drop or two of normal H_2SO_4. Add 0.1 N ferric ammonium sulphate (0.033 m.) using about 6 equivalents of iron for every equivalent of phosphate. In practice 1 c.c. of 0.1 N ferric alum will completely precipitate up to 0.020 milli-equivalent of phosphate in the sample. Add 0.1 N NH_4OH just to a full red to the indicator and filter. Transfer 20 c.c. of the filtrate to a silica beaker or platinum dish, add a drop of concentrated H_2SO_4, evaporate, and

ignite, continuing the analysis as outlined under method for blood. In the calculation, of course, due allowance is made for the aliquot taken.

Wohlbach, S. B., and Howe, P. R.: Intercellular Substance in Experimental Scorbutus. Arch. Path. and Lab. Med., January, 1926, i, 1:1.

In a paper with sixteen illustrations (microphotographs) a careful and extensive study is reported tending to demonstrate that scorbutus represents an inability of the supporting tissues to produce and maintain intercellular substances.

It is suggested that this failure is due to the absence of an agent common to all supporting tissues, which is responsible for the setting or jelling of a liquid product. Antiscorbutic substance is liberated in the destruction of tissues. The paper does not lend itself well to abstraction and should be read in the original.

Lippincott, L. S.: A Technic for Preparing Frozen Sections. Stain Technology, January, 1926, i, 1:39.

The method described has been very satisfactory.

1. Formalin 10 per cent overnight.
2. Place in distilled (not tap) water, freeze in a drop of distilled water and cut.
3. Transfer to distilled water and thence to a slide.
4. Drain and cover with 95 per cent alcohol.
5. Blot carefully on cover with very thin celloidin in equal parts of alcohol and ether.
6. Drain off celloidin and allow to set in air but do not dry.
7. Distilled water for two minutes.
8. Ripened Delafield's hematoxylin.
9. Two washings in clear distilled water.
10. 0.5 per cent equeous eosin two to five minutes.
11. 95 per cent alcohol till no further stain is removed (five minutes).
12. Drain off alcohol and flood with beechwood creosote. (Ten to fifteen minutes.)
13. Blot, mount in balsam, and examine.

Kalodny, A.: Hypernephroma of the Thyroid. Arch. Path. and Lab. Med., January, 1926, i, 1:37.

The second case in the world's literature is reported and described

BOOK REVIEWS

(Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.)

*Clinical Researches in Abdominal Disease**

THE author first emphasizes the value and importance of clinical research, as contrasted and correlated with pure laboratory research. "It is within the scope of the clinician to test with thoroughness the results achieved by work in the laboratory. It is a tribute to the clinician that the laboratory worker eagerly acclaims clinical results when they appear to support his experimental findings but quarrels with their accuracy when they do not confirm his views. It is also within the scope of clinical research to try various empirical methods and to furnish the results to the laboratory for explanation and elaboration. The clinical researcher must also record and compare clinical facts with the object of determining some questions which are inaccessible to laboratory methods of study."

The author discusses in detail the functions of the parietal peritoneum in localization of abdominal pain and zones of hyperesthesia or hyperalgesia, commonly known as Head's zones. Contrary to the usual statement, Dr. Cope finds that the location of the shoulder pains, whether anterior or posterior, *aids in the localization of diaphragmatic irritation.* The symptom on the other hand does not enable one to determine whether the pathology is above or below the diaphragm. Cope finds hyperesthesia in 59 per cent of his cases of acute appendicitis and in 47 per cent of all cases coming under the general category of the acute abdomen. The hyperesthesia may occasionally be left-sided in the former. The location of the hyperesthesia in the latter is not of distinct value in localizing the acute intra-abdominal lesion.

The Emotional Life of the Child†

THE experimental investigations of Helga Eng of Oslo into the emotional life of the child as compared with that of the adult have been translated by George H. Morrison. While the field of the author's experimental work is much more restricted than one would anticipate from the title, the results and conclusions are of unusual interest. She has aroused the various emotional responses in children and adults, following which she has traced changes in the pulse volume, arm volume, heart rate and the respiratory rate and depth. The author finds that spontaneous displeasure or depression curves are much less frequent in children than in adults, while spontaneous pleasure

*By Zachary Cope, B.A., M.D., M.S. (Lond.), F.R.C.S. (Eng.). Cloth. Pp. 148. Price $4.00. Humphrey Milford. Oxford Univ. Press. 1925.

†Experimental Investigations into the Emotional Life of the Child compared with that of the Adult. By Helda Eng, Oslo. Translated by George H. Morrison, M.B. (Edin.). Cloth. Pp. 243. Humphrey Milford. Oxford Univ. Press. 1925.

curves as well as pleasure curves resulting from external stimuli are more frequent. The work covers a wide range of emotions which cannot be reviewed in the space at our disposal.

On the basis of experimental evidence the author takes exception to the theory of James that we do not cry because we are sad but we are sad because we cry. She believes that the psychic processes and reactions more nearly control the somatic function than the reverse.

Among those psychologists who have made any intensive studies of the emotions with this or similar methods, we find nearly as many hypothetical conclusions as there are investigators. While the work probably will be subject to some criticism as other methods of study are developed, it now holds a high place in the literature on the subject.

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*Physical Chemistry in Biology and Medicine**

THE advancement of science is partly dependent upon the application of the discoveries in one branch to other branches. This volume is intended to indicate the application of recent and advanced work in physical chemistry to biology and medicine. It is not in any sense a textbook on the subjects covered but is intended for the research worker in biology and medicine, to whom it should prove a useful and rather comprehensive reference.

Within its fifteen chapters a résumé of all the recent work in this field is clearly presented.

The book is well arranged and well printed, and, though the subject is complex, the style is clear and readable.

Postmortem Appearances†

MORGAGNI, the founder of modern pathology, interested himself in gross pathologic lesions. With the passage of time interest has centered more and more in microscopic appearances, and this phase of the study has been stressed in the medical curriculum to such an extent that not infrequently one finds students who have the impression that pathology is always a question of microscopic appearance. The medical student learns to diagnose abnormal tissue changes with considerable accuracy, but we would find not infrequently that if he were handed a whole organ on which to make a gross diagnosis he would fail lamentably.

This weakness in our teaching system, which fortunately is being rapidly overcome, is an important one because the surgeon from the nature of his work must be a thoroughly trained gross pathologist. He must be able to recognize and interpret gross changes immediately, in so far as possible, during the operation, so that he may best decide upon the proper operative procedure.

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*Physical Chemistry in Biology and Medicine. By J. F. McClendon, Assistant Professor of Physiologic Chemistry, and Grace Medes, Assistant Professor of Physiologic Chemistry, University of Minnesota, Medical School. Cloth. 34 illustrations. 425 pages. Price $4.50. W. B. Saunders Company.

†Postmortem Appearances. By Joan M. Ross, M.B. B.S., (Lond.) M.R.C.S., L.R.C.P., with preface by E. H. Kettle, M.D. Cloth. Pp. 216. Price $2.50. Humphrey Milford. Oxford Univ. Press. 1925.

To a certain extent this little handbook fulfils the requirements of a reference manual on gross pathology. The reviewer feels that while it is laudable, indeed essential, to describe the changes in remote organs affected during the process of a local disease, such as carcinoma of the stomach, the value of the contribution would be increased if in each case more details were given in the description of the major local pathology.

The appendix contains useful information, such as the average dimensions of adult organs, weight of organs in the newborn, the date of ossification of the principal bones, and the ages of the eruption of the teeth, dates which are well known to every grandmother, but which the majority of doctors are unable to specify.

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*Development of Our Knowledge of Tuberculosis**

HISTORY is one of our greatest sources of instruction. An historical presentation, even one whose scope is as limited as that under review, cannot but be of tremendous interest, particularly when presented in an authoritative manner. Perhaps the most remarkable observation in historical reviews of this sort is how clearly some of our antecedents saw conditions and how epoch-making observations are often lost to sight for years, indeed centuries, to be rediscovered by another who later, to his chagrin, finds himself antedated.

Long before the Christian era the laws of Manu in India declared pulmonary consumption and swelling of the glands of the neck to be unclean, incurable diseases, and cautioned those who desired matrimony not to select a spouse from an unhealthy family, even though the family had good lineage and possessed great wealth.

In 1782 the laws of the kingdom of Naples required that the physician shall report a consumptive case when ulceration of the lungs has been established under penalty of 300 ducats for the first offense and banishment for ten years for repetition of it. Today we still experience difficulty in enforcing the reporting of tuberculosis.

But the chief interest in Dr. Flick's comprehensive review is in his description of the long, laborious, painstaking search for the truth as revealed in the writings of the leading thinkers through the centuries. Often the leaders of medical thought have been very far afield from the truth.

The review terminates with the beginning of the 20th century.

*Development of Our Knowledge of Tuberculosis. By Lawrence F. Flick, M.D., L.L.D. Cloth. Pp. 783. Price $7.50. 738 Pine St., Philadelphia, Pa. 1925.

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EDITORIALS

Appendicitis

EVEN after many years of study of appendicitis the problem of etiology remains. Ever and anon an investigator endeavors to show that the cause of the disease is an unusual organism and attempts to relegate B. coli to the background. But always, it seems, renewed bacteriologic investigations tend to prove that B. coli is, after all, the commonest invader of the appendix and the most important cause of appendicitis. If this is the actual state of affairs, inflammations of the appendix are rarely if ever of hematogenous origin. Aschoff is perhaps the most recent of the so-called authorities to refer appendicitis to unusual causes, and he believes that the streptococcus, either alone or associated with a slender, curved gram-positive bacillus, is the predominating cause. And yet McWilliams found that 57 per cent of 288 cases showed B. coli and only 8.3 per cent showed streptococcus alone.

Warren in a recent article reports the study of 100 appendices from cases in which a diagnosis of acute appendicitis had been made. Of the 100 specimens, only 66 could be diagnosed, by histologic methods, as actually acute. By means of careful bacteriologic methods the organisms invading the walls of the

diseased organs were studied and the results are shown in the following table. The figures apply only to the 66 acute cases.

	SEROSA	MUSCULARIS	MUCOSA	TOTAL
B. coli communis	14	17	18	18
B. coli communior	7	7	7	7
Streptococcus hemolyticus	5	5	5	6
Streptococcus hemolyticus + B. coli	9	9	9	9
Proteus vulgaris	4	4	5	5
B. pyocyaneus	4	4	4	4
B. pyocyaneus + B. coli	1	1	1	1
Streptococcus viridans	3	2	3	3
Streptococcus viridans + B. coli	1	1	1	1
Staphylococcus albus + B. coli	1	1	1	1
Pneumococcus type I	1	1	1	1
Streptococcus (nonhemolytic)	0	0	0	1
No growth	–	–	–	9

There were four cases of periappendicitis, two of which yielded Pneumococcus type I, one Streptococcus viridans, and one the gonococcus.

In 16 cases (in the series of 100) composing the group of "healing" appendicitis, one showed Streptococcus viridans, and one B. coli, both present in the serosa and not in the muscularis. In the remainder of the cases, no growths were secured. This is the group of "healed appendicitis."

The study of this series of cases brought up the problem of tissue diagnosis of surgical specimens as it relates to the appendix. It has been more or less customary for a hospital pathologist to use a very cumbersome series of designations for diagnostic purposes, as, for instance, acute appendicitis, subacute appendicitis, chronic appendicitis, adding to each of these such descriptive words as suppurative, catarrhal, fibrous or fibroid, etc., according as certain appearances in the gross specimen appeal to him. It is obvious that such a nomenclature must vary widely in its actual meaning, and it is also obvious that the diagnosis "chronic appendicitis" is apt to cover not a few surgical misjudgments, and that the term "acute appendicitis" is overworked both by surgeon and pathologist. In the group of 100 cases selected by Warren as *acute* only 66 per cent turned out to be actually acute.

Therefore, as a result of the combined bacteriologic and histologic study Warren suggests the following as the minimum requirements for a diagnosis of the various conditions:

"For acute appendicitis, pus in the lumen and some evidence of inflammation in the wall;

"For healing appendicitis, fairly numerous eosinophiles in the muscularis, also foci of lymphocytes;

"For healed appendicitis, focal collections of lymphocytes in the muscularis or serosa, either inside or outside lymphatics.

"The diagnosis chronic appendicitis is not made. The dividing line between acute and healing appendicitis is not very clear cut, but the lesion is called healing when there is a predominance of eosinophiles over polymorphonuclear leucocytes in the exudate and evidence of proliferation of the fibroblasts. The presence of eosinophiles in the mucosa is considered normal."

These requirements for diagnosis are surely helpful.

—*P. G. W.*

Toxin-Antitoxin Immunization as a Source of Anaphylaxis

PHARMACOLOGY recognizes, in addition to the predominant therapeutic effects of drugs, untoward by-effects not always desirable. It is possible that the practical application of immunology to the prevention of disease must receive a similar consideration.

Stewart,[1] in a recent communication, calls attention to toxin-antitoxin immunization against diphtheria as a factor responsible for an increasing number of individuals, particularly children, sensitized to horse serum and hence liable to anaphylactic reactions.

The increasing use of scarlatinal antistreptococcic serum may serve to increase still further the number of sensitized children so that this possibility must ever be borne in mind when necessity arises for the administration of serum to children as a means of prophylaxis or therapeusis.

Were the anaphylactic manifestations simply those of the usually readily-controlled "serum sickness" the matter would be of relatively minor importance. Stewart, however, encountered seven cases in which the administration of serum for scarlatinal prophylaxis in children previously actively immunized against diphtheria eight to eleven months before was followed by reactions of marked intensity which assumed an alarming degree of severity.

Although no deaths resulted, the occurrence serves as a warning to use care and to remember the possibility.

Active immunization of the child population against diphtheria is so important that its neglect cannot be contemplated. It would seem advisable, however, that studies be directed toward the evolution of some method whereby the production of marked sensitization may be minimized if it cannot be avoided.

It has been suggested by Kolmer that it might be possible to prepare an ox-serum diphtheria antitoxin which could be used for prophylaxis, reserving the horse serum antitoxin for treatment. Some such procedure would, at least, lessen the likelihood of anaphylactic reactions.

Larson and numerous associates[2, 3] have recently presented the results of investigations directly applicable, and so applied by them, to this problem.

During the course of studies upon the effect of surface tension depressants upon bacterial toxins, it was found that when a solution of sodium ricinoleate was brought into contact with a bacterial toxin the toxin was "detoxified." As a result, several hundred times the normal lethal dose could be injected into animals without ill effect.

It was further observed that such detoxified toxins suffered no alteration in their antigenic properties, that the production of immune bodies followed their injection, and that, because of the large dosage possible, immunization occurred very rapidly.

The assumption is that the action of the soap is an adsorption phenomenon and that protection from the toxin occurs by the adsorption of a layer of soap over the entire toxin molecule.

These observations have been applied to the active immunization of human beings, children and adults, against diphtheria and scarlet fever.

It is claimed that when using such detoxified toxins, large doses may be given without either local or general reactions and followed by the production of an active immunity within a short space of time.

If these results are confirmed, a very definite advance will have been made in that active immunization will be possible without an attendant sensitization occurring.

The problem merits serious and extensive study in order that active immunization and the specific serum treatment of disease may not become a source of alarm to the laity because of severe reactions consequent upon the use of these valuable and, indeed, indispensable weapons in the treatment and prevention of disease.

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—*R. A. K.*

Measles

WITH the advent of modern and intensive studies of disease, in particular as well as in general, new concepts take the place of old throughout the entire field of medical practice.

Particular attention has been paid to many of the so-called "children's diseases" which, in olden days, were regarded as necessary and almost unavoidable evils, and many of these, through the acquisition of definite information regarding their etiology and prophylaxis, have more or less been brought under control.

Measles, however, perhaps because sufficiently intensive studies have yet to be made, remains, economically, one of the most important of the diseases of early life.

The deaths from measles in childhood are numbered among the thousands yearly, and the death rate in the first five years of life from bronchopneumonia, so frequently associated with this infection, reaches a total of nearly 50 per cent. In addition, there are no readily available statistics to indicate the degree or extent of the sequelae of measles, such as tuberculosis or conditions affecting the eyes, ears, and other structures.

The disease seems ubiquitous. There is no part of the globe where it does not occur, and it seems to have been known from time immemorial, being held identical with the Sanskrit "masura" (spots).

Definite knowledge of the nature of the etiologic agent is so far lacking, other than that it is present in the secretions of the nose and throat and perhaps the blood, during the first stages of the disease.

Tunnicliff and Moody[1] have described a gram-positive, anaerobic diplococ-

cus obtained from the blood which grew aerobically in the second generation, producing green colonies on an aerobic blood plate.

This organism is stated to have agglutinated with the blood of measles cases from the third to the eighth day after the appearance of the eruption, opsonins also being formed.

While these investigators believe that inoculation into rabbits, guinea pigs, and monkeys was followed by symptoms comparable to measles in the human being, and that these results were due to the coccus in question, an atmosphere of doubt arises from their further statement that "it is possible that the diplococcus possesses the selective power of carrying the specific virus of measles."

Further corroboration of this work has not, apparently, been reported.

The experimental production of measles in the lower animals is a matter of some difficulty, the characteristic picture of the human reaction not having been reproduced.

Scott and Simon[2] review the literature of rabbit infections and report experiments indicating that rabbits react to measles with a marked elevation of temperature and an amphophilic leucopenia, the latter being the more characteristic manifestation in these animals.

The same observers[3] report the occurrence of protective bodies in the blood of rabbits after inoculation with blood or nasopharyngeal secretions from cases of human measles.

There seems to be no doubt that the etiologic agent is present in both the nasopharyngeal secretions and the blood and that protective bodies appear in the blood stream.

Little is known, however, of the pathology of uncomplicated measles, because death is generally due to the accompanying bronchopneumonia or to later complications.

In the cases reported by Denton[4] in which death occurred so soon after onset (within a few hours) that the lesions were probably due to uncomplicated measles, the primary lesions were mainly in the lower trachea, bronchi, and contiguous lung and were in the nature of an acute, congestive inflammatory reaction without distinctive characteristics.

Because of the lasting immunity following attacks, convalescent serum has been used with encouraging results in its treatment and prophylaxis.

Regan[5] reports that the symptomatology was much modified in immunized individuals later developing the disease, and McNeal[6] believes this to be an efficacious prophylactic measure.

Zingher[7] has used the method rather extensively and has shown that either plasma, serum, or whole blood (citrated or noncitrated) may be used, the resulting immunity persisting from thirty to forty-five days.

Weaver and Crooks[8] believe that prophylaxis through the injection of convalescent serum should be practiced in children under the age of five.

The method, while of undoubted efficacy, is handicapped by the difficulty of obtaining a sufficient supply of serum and the effective specific prophylaxis and treatment of this disease awaits the demonstration of its specific agent.

The problem merits persistent and organized study.

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—*R. A. K.*

CORRESPONDENCE

19th December, 1925.

To the Editor:

In connection with the paper by Dr. Anderson on the "Use of a Suction Apparatus for Bleeding Rabbits" which appeared in the JOURNAL OF LABORATORY AND CLINICAL MEDICINE for November, 1925, it may be of interest to note that a similar, indeed almost identical, apparatus was introduced for the same purpose in the Wellcome Tropical Research Laboratories at Khartoum prior to 1911. An account of it, with illustrations of the apparatus, will be found in the Fourth Report of these Laboratories, Vol. A., p. 107. I may say the apparatus was primarily introduced for the purpose of preventing contamination of blood agar tubes, which is very likely to occur in a climate like that of Khartoum, but the arrangement was also found of use in the manner indicated by Dr. Anderson of Toronto, i. e., a larger amount of blood could be obtained from each rabbit by this method than was possible by any other means.

Andrew Balfour,
Director, London School of Hygiene
and Tropical Medicine.

Jan. 25th, 1926.

To the Editor:

The publication referred to in Dr. Balfour's letter is not available for perusal in the city of Toronto, and I am therefore unable to verify the claims regarding priority of publication of a suction apparatus for bleeding small animals. I am, however, pleased to learn that Dr. Balfour endorses the use of an apparatus of this type.

C. M. Anderson,
Director of Laboratories, Ontario
Department of Health.

The American Society of Clinical Pathologists

The Society maintains a Service Bureau for its members. Any clinical pathologist wishing to make a change should communicate with the Secretary.

Kindly report any change of address to the Secretary.

The Journal of Laboratory and Clinical Medicine

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CLINICAL AND EXPERIMENTAL

STUDIES IN TOXICOLOGIC CHEMISTRY*

I. THE DETECTION OF THE OPIUM ALKALOIDS BY SELENIOUS-SULPHURIC ACID: THE SPECIFICITY OF THIS REAGENT FOR THE PHENOLIC GROUP

BY VICTOR E. LEVINE, OMAHA, NEBR.

WHEN one runs through the pages of a text devoted to toxicology, he is at once struck by the very great number of tests that have been devised for the toxic compounds usually met with in cadaveric material. These tests have been at times offered as legal evidence. Yet, in spite of their importance in forensic medicine, many of these have been inadequately investigated and have not been closely scrutinized as to their limitations and as to their specificity. To cite an example: A Portuguese physician, Urbino de Freitas, was accused of having poisoned three of his wife's nephews and of having brought about the death of one of them by poison given in enemata.[1] The case was tried in 1893. The symptoms were those caused by opium in part only.

The experts for the prosecution reported the presence of morphine, narcein and delphinin in the tissues. They relied for their evidence as to the presence of these alkaloids upon a positive test with iodic acid, with Froehde's reagent, and with Lafon's reagent consisting of a mixture of selenious and sulphuric acids. It is now well known that neither the reduction of iodic acid nor the reduction of the molybdenum compound in Froehde's reagent is specific for the alkaloids mentioned, since hosts of organic compounds of biologic and nonbiologic occurrence also have the power of reducing. The third reagent is highly recommended by Mecke, Autenrieth and others, and is considered specific for the alkaloids of the morphine group. In the course of this research we shall prove that the selenious-sulphuric reaction, while being very sensitive for these

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alkaloids, is also given by phenols. The opium alkaloids, therefore, react by reason of the phenolic group or groups present in the molecule. In the light of forensic medicine, it is of great importance to eliminate the presence in the tissue of such phenols, as phenol, cresol, tyrosin, hydroquinone, pyrocatechin, adrenalin and others, before ascribing a positive reaction to the presence of the opium alkaloids.

The first one to report the use of selenium as an alkaloidal reagent was Brandt. In 1875 he described a number of reactions obtained by a reagent containing concentrated sulphuric acid to which had been added a small quantity of selenic acid. Morphine and narcotine yielded characteristic color reactions. Morphine gave at first a bluish green, then a grass green, which quickly changed into reddish yellow, red and brown. Narcotine gave a blue color, which finally became green.

In 1885 Lafon reported a color reaction for morphine and codeine involving the use of a salt of selenious acid. Upon adding these alkaloids to ammonium selenite dissolved in sulphuric acid he obtained a magnificent green color. Sodium selenate he found also effective, although the color did not appear as quickly, nor was it as intense as that obtained with the selenite.

In 1891 Ferreira da Silva examined a number of alkaloids with the Lafon reagent and found that it permitted the characterization of berberin, eserin, narcein, narcotine, papaverine and solanin. Mecke, in 1899, reported experiments with an alkaloidal reagent consisting of 0.5 gram selenious acid dissolved in 100 grams of pure concentrated sulphuric acid. He recommended its use in the detection of the alkaloids found in opium. His results with these and with other compounds are given in tabular form.

TABLE I

ALKALOID	REACTION IN THE COLD	REACTION ON HEATING
Acontin	colorless	dark brown violet
Apomorphine	dark blue violet	gradually turns dark brown
Atropine	colorless	almost colorless
Brucin	yellowish red	citron green
Caffeine	colorless	colorless
Cocaine	colorless	rose yellow
Codeine	blue, quickly changing to emerald green, and later to persistent olive	steel blue, then brown
Colchicin	intense citron yellow	yellow-brown
Conium	colorless	colorless
Delphinin	deep brown	brown
Digitalin	yellow, changing quickly to red and gradually paling	bluish violet, then brown
Morphine	evanescent blue, then intense blue, green to persistent olive green	brown
Narcein	faint greenish yellow, then violet	dark violet
Narcotine	greenish steel blue, later cherry red	
Nicotine	yellowish	yellowish
Papaverin	greenish, dark steel blue, then deep violet resembling methyl violet	intense dark methyl violet
Physostigmine	brownish yellow	weakly brown
Picrotoxin	almost colorless	yellowish brown
Quinine	colorless	dark brown
Solanin	gray-brown	gray-brown
Strychnine	colorless	colorless
Thebain	deep orange, gradually paling	dark brown
Veratrin	citron yellow, later olive green	brownish violet

Table I shows that Mecke's reagent produces characteristic color effects with the opium alkaloids, morphine, codeine, narcein, narcotine, papaverin, thebain and apomorphine. To these we may add dionin or ethyl morphine and heroin or diacetyl morphine, for we have found that these two morphine derivatives yield an evanescent blue, changing quickly to the characteristic emerald green.

In a number of comparative tests Mecke found his reagent more sensitive than many of the older ones. The characteristic reaction appeared immediately in the cold. In practical work, which involved the testing for the presence of alkaloids in tissue, the heat necessary for extraction processes gives rise to *brown impurities, which often hide the color effects produced by reagents.* He observed, however, that the color which the selenium reagent produced was always noticeable in the brown extraction mixture. According to Morgulis and Levine, 0.01 milligram of morphine, equivalent to 0.0025 milligram of morphine sulphate, yields a strong positive reaction, while 0.005 milligram of the alkaloid equivalent to 0.0125 milligram of the sulphate gives a faint but recognizable reaction.

The colors observed for brucin, colchicin, delphenin and veratrin are no doubt due to the sulphuric acid in the reagent. Concentrated sulphuric acid alone is said to give yellow, orange or brown colors with veratrin, colchicin, delphenin, gelsemin and lobelin. Veratrin in the presence of concentrated sulphuric acid assumes an intense yellow color and finally dissolves to a yellow solution. This soon changes to an orange shade with a strong greenish fluorescence shown by reflected light. The separate alkaloids of veratrum album and viride show changes in sulphuric acid, varying from red to violet or green.

The Lafon or Mecke reagent is not specific for the alkaloids of the opium group. Table II lists a number of compounds that give a positive reaction. The reagent employed consists of 0.5 per cent selenium dioxide or 0.75 per cent sodium selenite in pure concentrated sulphuric acid.

As seen from the results, phenolic compounds in contact with a solution of 0.5 per cent selenium dioxide or 0.75 per cent sodium selenite in pure concentrated sulphuric acid yield a pale green, olive green, bluish green, or purplish green color. Sometimes several shades are observed simultaneously. More often one shade gives way to a succession of shades. In addition to the characteristic color produced by the phenol, there is often observed the concomitant decomposition of the selenious-sulphuric acid reagent with the formation of a brick-red or brown colloidal solution, suspension, or precipitate of free selenium. The characteristic coloration produced by the *positive-reacting compound is at* first so intense that the brown-red or brick-red selenium does not mask it. Many organic compounds, notably carbohydrates, reduce the selenious-sulphuric acid reagent with the formation of free red selenium. The characteristic colors formed in the presence of phenols, however, are not permanent. They disappear on standing and are subsequently replaced by the red or brown selenium tint. The direct addition of water to the greenish reaction mixture results in the rapid fading of the green and the appearance of a reddish or brownish coloration.

The characteristic color reaction may be explained on the basis of the fact

TABLE II

MONOPHENOLS	
Phenol	Grass green. The intensity of the color begins to diminish after 2½ hours.
o-Cresol (methyl phenol)	Dark green, fading in about 1 minute to brown with a pink tinge.
m-Cresol	Deep emerald green. The color is more intense after 1 hour. Very little of the original color remains after 2 hours.
p-Cresol	Fleeting olive green, which can be noticed about the edges of the container, finally changing to brown.
o-Xylenol (dimethyl phenol)	Reddish brown, changing in about 1½ minutes to reddish purple, and in 2 minutes to deeper red shade.
m-Xylenol	Deep red, changing to reddish brown in 1 hour.
p-Xylenol	Purplish, changing to characteristic green and fading in about 12 minutes.
Thymol	A beautiful play of colors. Yellowish, bluish, purplish, cherry red and bright green colorations are observed in succession or simultaneously. The color display is very distinct at the sides of the crucible.
Carvacrol	Bright green on sides and bottom of crucible. Reddish brown also appears simultaneously with the green. The coloration persists 3 hours, especially along sides of crucible.
α-Naphthol	Deep emerald green, giving way in 20 minutes to muddy rust brown precipitate.
β-Naphthol	Very slight indication of fleeting olive green, which is replaced by brown.
MONOPHENOLS WITH ALCOHOL GROUP	
Diathesin (o-hydroxybenzyl alcohol)	
MONOPHENOLS WITH ALDEHYDE GROUP	
Salicylic aldehyde (o-hydroxybenzaldehyde)	Yellowish green giving way to crimson. The greenish tinge disappears after 1 hour.
MONOPHENOLS WITH CARBOXYL GROUP	
Salicylic acid (o-hydroxybenzoic acid)	Very faint trace of green which disappears immediately.
Aspirin (acetyl salicylic acid)	No color changes in the reaction mixture.
Tyrosine	Brown and olive green, giving way to a bluish green and then to an intense blue, finally disappearing after 5 hours.
β-oxynaphthoic acid	Dark brown with tinge of olive green and greenish blue, which persists after three hours.
ESTERS AND SALTS OF PHENOLIC ACIDS	
Oil of wintergreen (methyl salicylate)	Fleeting light green. Very faint green may still be observed after 1 hour.
Salol (phenyl salicylate)	Light yellowish green, changing to grass green in 1 minute, later to characteristic bluish green. This color fades in about 45 minutes, giving way to pink.
Sodium salicylate	No change in reaction mixture.
MONOHYDRIC PHENOLS WITH HALOGEN	
Di-iodothymol	No change.
NITRATED MONOHYDRIC PHENOLS	
o-Nitrophenol	No change.
p-Nitrophenol	No change.
Dinitrophenol	No change.
Picric acid	No change.
MONOHYDRIC PHENOLS WITH AMINO GROUP	
p-Amidophenol hydrochloride	Purple color with a slight greenish tinge. With sulphuric acid alone a bluish tinge, which disappears almost immediately, giving place to a black color.
Photol (mono-methyl p-amidophenol hydrochloride)	Turns pink in 2 minutes; changes to reddish brown with slight purple tinge in 4 minutes.
Metol (methyl-amino-cresol sulphate)	Faint pink appearing after ½ minute; changes to cherry red. With sulphuric acid alone no characteristic reaction.
Amidol (2, 4 diamino phenol sulphate)	Dark purple changing to blue, and finally changing to red within 18 hours. With sulphuric acid alone a bluish color develops.
MONOHYDRIC PHENOLS WITH NITRO AND AMINO GROUPS	
Picramic acid (amino-dinitro phenol)	No reaction.

TABLE II—CONT'D

ETHERS OF MONOHYDRIC PHENOLS	
Anisole (methyl phenyl ether)	Dark green persisting after 3 hours.
Phenetole (ethyl phenyl ether)	Emerald green at bottom of crucible. Surrounding liquid is pink. After an hour the green is still to be observed, but begins to fade after 3 hours.
ETHERS OF MONOHYDRIC PHENOLIC ACIDS	
Anisic acid (p-methoxy benzoic acid)	Grass green giving way to brown coloration in about 2 hours.
DIHYDRIC PHENOLS	
Pyrocatechin (o-dihydroxybenzene)	Beautiful emerald green, persisting after 18 hours. This color gives way to an intense blue on the second day and to a blue precipitate on the third day.
Adrenalin (o-dihydroxyphenol hydroxyethyl methylamine)	Emerald green, giving way within 10 minutes to red brown.
Resorcin (m-dihydroxybenzene)	Yellowish brown, giving way to red brown in one hour.
Orcinol (methyl resorcinol)	Grass green, changing to deep olive green almost immediately. The color persists but takes a slight brownish tinge in 15 minutes. A dark brown remains to be seen at end of 45 minutes with only a slight tinge of green.
Hydroquinone (p-dihydroxybenzene)	Brown and purplish coloration with a tinge of green. A slight greenish tinge persists in the purplish brown after 1 hour.
ETHERS OF DIPHENOLS	
Guaiacol (monomethyl ether of pyrocatechol)	Dark olive green persisting after 2½ hours.
Eugenol (allyl 4, 3 guaiacol)	Yellowish green, giving way to crimson after 1 hour. The sides of the crucible show a purplish coloration.
ETHERS OF DIPHENOLS WITH ALDEHYDE GROUP	
Vanillin (m-methozy-p-hydroxy benzaldehyde)	Yellowish green. Slight trace of green is still present after 1 hour.
Piperonal (heliotropine) (anhydride of vanillin)	Orange and bright green. After 1 hour the green increases in intensity.
ETHERS OF DIPHENOLS WITH CARBOXYL GROUP	
Vanillic acid (m-methoxy p-hydroxy benzoic acid)	Slight tinge of yellowish green, fading completely after 1 hour.
TRIHYDRIC PHENOLS	
Pyrogallol (1, 2, 3 trihydroxy benzene)	Brownish black color, becoming almost black in 5 minutes.
Phloroglucin (1, 3, 5 trihydroxy benzene)	Beautiful emerald green, fading within 30 minutes.
TRIHYDRIC PHENOLS WITH CARBOXYL GROUP	
Gallic acid (3, 4, 5 trihydroxybenzoic acid)	Beautiful emerald green persisting after 10 minutes.
Tannic acid (digallic acid)	Olive green tinge at first, then to dark brown. At end of 24 hours the reaction mixture is reddish.
Dermatol (basic bismuth gallate)	Dark olive green changing to blue in 3 to 4 minutes, and changing back to green after 20 minutes.
GLUCOSIDES YIELDING PHENOLS ON HYDROLYSIS	
Arbutin (glucose + hydroquinone)	Yellowish brown changing to dark brown in 1 minute.
Esculin (glucose + 4, 5 dihydroxy coumarin)	Yellowish green disappearing in 1 hour.
Phloridzin (glucose + phloretic acid + phloroglucin)	Olive green, becoming deep bluish green and finally giving way to a red brown precipitate.
Salicin (glucose + o-hydroxy benzyl alcohol)	Beautiful cherry red. The same color reaction takes place with sulphuric acid alone.
Saponin	Light green changing immediately to brown. Slight green color at edges, which persists for 1 hour.

that selenious acid is a compound which is readily reduced, yielding oxygen. The phenols reduce the selenious acid and they in turn undergo oxidation with the formation of chromogenic derivatives. The appearance of the red or brown-red color after the primary color reaction is due to the selenium released in the course of the decomposition of the selenious acid. The view that the green color is caused by the free selenium dissolving in the concentrated sulphuric acid to form a green solution of selenosulphur trioxide ($SeSO_3$) is untenable, since lactic acid, glucose and other carbohydrates react with the selenious-sulphuric acid to give only a red or brick-red coloration characteristic of elemental selenium.

From the fact that the selenium reagent gives a positive reaction with phenols, it seems plausible to postulate that the phenolic hydroxyl in the morphine alkaloids is responsible for the reaction described by Lafon, da Silva, Mecke, and others. Morphine contains two hydroxyl groups, one of which is phenolic. Codeine is a phenolic ether, methyl morphine. Dionin is ethyl morphine; heroin, diacetyl morphine. Apomorphine is made from morphine by the removal of a molecule of water as a result of dehydration with concentrated hydrochloric acid. Thebain has two methoxy groups, narcotine and narcein three, and papaverin four. The characteristic reaction is given by free phenols as well as those in which the hydrogen of the phenolic hydroxyl is substituted by an alkyl radical. In this respect the reagent differs from ferric chloride, which does not react with phenolic ethers like codeine, anethol and phenetol. The sulphuric acid of the selenium reagent decomposes the methoxy group with the liberation of the free phenol.

Besides the phenolic alkaloids of the opium group, berberine also yields a distinct reaction. Ferreira da Silva observed that it gave a yellowish green, which turned to brown, then to rose at the edge of the reaction mixture and violet in the middle. Berberin contains two methoxy groups. Quinine has one hydroxy and one methoxy group, but it does not give the phenol reaction. The neighboring groups in the molecule probably interfere with the reaction as they do in the case of some of the nonreacting phenols.

The selenious-sulphuric acid reaction which we propose as a specific reaction for the phenol group is one of wide applicability. This reaction is positive with monophenols and monophenolic ethers, or monophenols with such substituents as halogen, amino group, aldehyde or carboxyl group, with diphenols and their derivatives, with triphenols, with phenolic glucosides, and phenolic alkaloids. The reaction besides enjoying wide applicability is also very delicate. Only a very minute quantity of the solid substance, even a fraction of one crystal, is necessary for a positive response. A small quantity of dry material, preferably in powdered form, is placed in a small white porcelain crucible and the selenious-sulphuric acid is allowed to flow drop by drop down the sides of the crucible. About five drops are added. Usually the first drop making contact with but a minute quantity of the substance in the crucible brings out very vividly and almost instantaneously the characteristic green coloration. It is advisable to rotate the crucible sideways, as very often a play of colors is observed at the edge of the mixture. If the phenol is dissolved in water or in an organic solvent it may first be evaporated to dryness on the water-bath. A drop or two

of sulphuric acid may be added during the evaporation to prevent any rapid oxidation of the phenol.

The test proposed also lends itself to a beautiful zone reaction. The phenol may be dissolved in an organic solvent, which in itself does not decompose the selenious-sulphuric reagent with the liberation of selenium, or the aqueous solution of the phenol may be extracted from such solvent. Methyl alcohol, ethyl alcohol, ether and caprylic alcohol are not available because of the decomposition they induce in the selenious-sulphuric acid mixture. Amyl alcohol brings about very slight decomposition and chloroform none whatever. Both of these compounds are excellent solvents for morphine. The addition of selenious-sulphuric acid to an amyl alcohol or, preferably, chloroform solution of a phenol results in the formation of a beautiful emerald green ring at the point of junction of the two immiscible liquids. The green zone lies as a colored layer on the surface of the sulphuric acid and finally gives way to solid green particles, which float at the juncture, slightly submerged under the selenious-sulphuric acid.

The color reaction is sometimes influenced in its course by the substituent groups on the benzene ring carrying the phenolic hydroxyl. Nitrating the phenol abolishes the reaction, for o-nitrophenol, p-nitrophenol, dinitrophenol, trinitrophenol (picric acid) and amino dinitrophenol (picramic acid) fail to respond to the test. The presence in the molecule of nitro groups, derived from such a strong oxidizer as nitric acid, exerts a retarding influence upon the reducing capacity of the phenol. The presence of an aldehyde or carboxyl group on the benzene nucleus also inhibits the reaction, for salicylic aldehyde, salicylic acid, vanillin and vanillic acid yield exceedingly faint responses. Acetyl salicylic acid proves entirely negative. Esters of salicylic acid, however, react strongly. While thymol responds in a most vigorous way, di-iodothymol has no effect on the reagent.

Of all the phenols, pyrocatechol gives the most durable and the most vivid color. In testing for selenium in impure sulphuric acid it would be advisable to employ pyrocatechol instead of codeine phosphate, recommended by E. Schmidt and also by Schlagdenhaufen and Pagel.

The color reaction also admits of the differentiation of α-naphthol from β-naphthol. The former yields a deep emerald green, persisting for twenty minutes, while the latter gives a very slight indication of a fleeting olive green. The selenious-sulphuric acid reagent is also useful in distinguishing acetic anhydride from glacial acetic acid. The addition of the anhydride to the reagent results in the formation of elemental selenium, which appears as a brick-red collodial solution or precipitate. Glacial acetic acid, however, is not affected by the reagent.

CONCLUSIONS

1. Selenious acid (or its alkali salts) dissolved in pure concentrated sulphuric acid forms a colorless reagent yielding a characteristic color reaction specific for the phenol group.

2. The color test is extremely sensitive and possesses wide applicability. It is given by monophenols, monophenolic ethers, monophenols with halogen,

amino, alcohol, aldehyde or carboxyl group, diphenols and their derivatives like ethers, etc., triphenols, phenolic glucosides, and phenolic alkaloids.

3. The intensity of the reaction is modified by the presence of various substituents besides the phenolic hydroxyl in the benzene nucleus. The presence of one or more nitro groups interferes with the reaction completely.

4. The selenious-sulphuric acid reagent of Mecke, Lafon and others gives the reaction characteristic of the morphine alkaloids because of the presence of the free phenolic hydroxyl or of one liberated by the interaction of sulphuric acid with one or more methoxy groups present.

5. Mecke or Lafon's test is not distinctive of the morphine alkaloids. In toxicologic examination of tissue it must be kept in mind that phenol, p-cresol, tyrosine, pyrocatechin, hydroquinine and adrenalin are phenolic compounds of biologic occurrence. A positive test is of no absolute significance, but a negative test is important as a conclusive indication of the absence of the morphine alkaloids.

6. The selenious-sulphuric acid reagent also serves to differentiate α-naphthol from β-naphthol, and glacial acetic acid from acetic anhydride.

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THE EFFECT OF ATROPINE AND THE RÔLE OF THE INVOLUNTARY NERVOUS SYSTEM IN INSULIN ACTION*

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FORMER studies[1, 3] of the authors, which are published in several articles, have dealt with the effect of insulin after different methods of administration. It has been found that there is a striking difference between the effect of insulin when like amounts are injected intradermally, subdermally or intravenously. The effect of intravenous injection lasts only for a short time and it has been known since the first publications of Banting and Best[2] that the subcutaneous administration is even more effective than the intravenous method, and, consequently, it has since been the method of choice in diabetic treatment.

Our work was started with the idea of finding more definite data about the clinical action of insulin, based on the knowledge that differences in the effect of the drug manifest themselves when different body tissues are used for injection. Though it may be stated that an injection of a nonspecific albumin solution, such as Aolan, acts more intensively if it is made into the skin than if it is injected subdermally, yet, if the dosage of such nonspecific agents is diminished to very small amounts, a stage is reached in which only injections within the skin are effective, while subdermal and intravenous injections of equal amounts do not show any measurable effect.

This effect of nonspecific agents can be measured by the effect on the involuntary nervous system, more particularly on its parasympathetic part, stimulation of which leads to a sudden displacement of the leucocytes in human beings. This displacement is noticeable in a peripheric leucopenia of a short duration, a symptom of which cannot be shown in the animal body because of the differences in the skin of animals and human beings.

With this knowledge in mind, we thought first that different kinds of injection of insulin with their different effects might lead to a more intimate knowledge of insulin action. Furthermore, we believed that the same explanation as mentioned above, which has shown the close relationship of the skin to the involuntary nervous system, might lead to the discovery of new facts concerning the action of insulin.

In our first publications we have demonstrated that like amounts of insulin have a larger effect if administered intradermally than if administered subcutaneously. These findings have been obtained from differences in the blood-sugar lowering effect of insulin in a series of animals which were used, after a short period of rest, for the different methods of administration of insulin, so that we could base our results upon the difference in different animals as well as in the same individual.

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How this difference occurred has not yet been explained. It will be the subject of this paper to describe further studies made for this purpose. As has been shown in the foregoing articles, as far as animals are concerned, the blood-sugar lowering effect of insulin begins almost at the same time and reaches almost similar levels. In some animals a lower level is reached earlier by the intradermal than by the subdermal mode of administration at the two-hour test. After the second hour a very noticeable difference in the blood-sugar curves showed a longer lasting and more intensive effect of the intradermal mode of administration. This may be demonstrated by two comparative curves giving the average of ten curves of the effect of the intradermal and subdermal modes of administration. The animals used were the same in both tests.

In explanation of this difference it seems reasonable to consider the difference of the tissues and other absorption properties as contributing causes to the differences in insulin action. We know that resorption from the skin takes

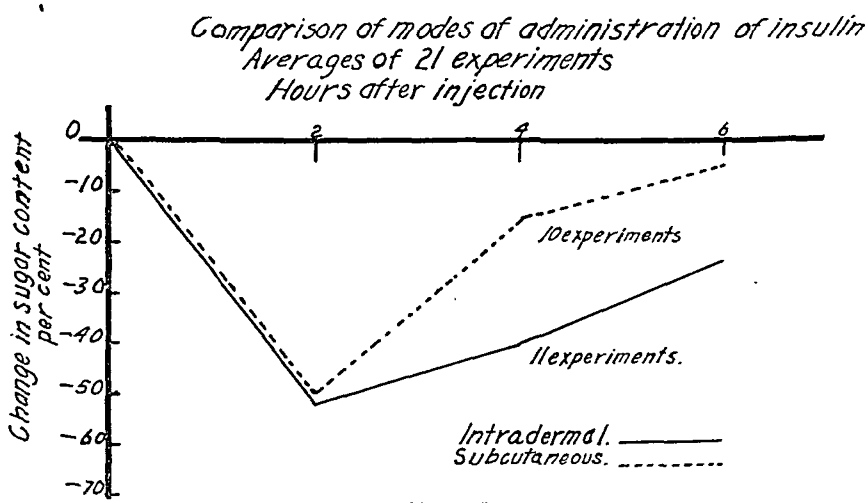

Chart I.

longer than from the subdermal tissues, i.e., insulin or any other substance injected into the skin will be present in the circulation later than after injection of the same into the subdermal tissues. This has been confirmed by the recent paper by Kasahara.[4]

Furthermore, it has to be considered that the longer the route of resorption, the more of the effective part of the absorbed agent will be lost by adsorption. These considerations show that in our experiments, insulin injected into the skin must reach the circulation later and in smaller quantities than the subdermally and the intramuscularly injected dose.

Moreover, we have to consider that during a given period smaller amounts of insulin reach the circulation from the skin than from a like injection into the subdermal tissues, thus showing that during this time, for instance, over a period of two hours after the injection has been performed, a smaller amount of insulin must be present in the circulatory system from the intradermal insulin deposit than from a like subdermal deposit.

All this does not agree with the observation that the measurable effect of

insulin after two hours is the same after subdermal and intradermal injection. Furthermore, it cannot be understood that, with definitely smaller amounts present in the circulation, the same or a lower blood-sugar content is really obtained, if one considers insulin active only after entering the blood stream. Experience shows that the effect in the circulation depends closely upon the dosage present. Thus small amounts injected intradermally must have a small effect and large doses a greater effect.

We have then, in brief, this condition: With the intradermal injection a larger amount of sugar is metabolized than after a subcutaneous injection of a like quantity of insulin. Yet, at any given time, there is, in all probability, a lower concentration of insulin in the circulatory system after intradermal than after subdermal injection. This leads us to believe that from our experiments we cannot conclude that the actual blood-sugar lowering effect depends entirely upon the amount of insulin present and active in the circulation. On the contrary, we have to assume that there must be some other way of insulin action in addition to the distribution of the drug by the circulation. This leads to the only possible conclusion that a postponed absorption produces a larger effect than an accelerated entrance into the circulation by subcutaneous administration. Thus the previously described findings of our experimental work in rabbits indicate that there must be another route of insulin action than the well-known effect by distributing the drug throughout the circulation which then acts in connection with the body tissues (muscles and liver) in reducing the blood-sugar level.

The question then arises as to how this other action is possible. We have mentioned in the beginning of this paper that from research on the action of nonspecific agents which has been confirmed by many authors, it has been found that the skin is closely related to the involuntary nervous system and injections into the skin always show a nonspecific reaction of the involuntary nervous system, consisting of a parasympathetic overbalance of the splanchnic region, while the periphery is overbalanced in the contrary direction. We cannot go into a more detailed description of these findings which may be found in the respective publications, but the striking parallelism between nonspecific agents and their action and insulin and its blood-sugar lowering effect, depending upon whether injected within the skin or under the skin, particularly called our attention to the fact that the involuntary nervous system may play an important part in the action of insulin if injected intradermally. Such an action of the involuntary nervous system may provide the missing link in the explanation proposed above.

We have stated that there must be a route of insulin action other than that by distribution of the hormone by the circulation, and now the more definite question arises: Does the involuntary nervous system represent this missing link, and is it possible to demonstrate this by definite experimental observation?

Two routes were feasible for this work. In former work with nonspecific agents[5] the involuntary nervous system has been interrupted by pharmacodynamic substances as well as by the knife, and in both cases the action was entirely cancelled, showing that the involuntary nervous system represents a "conditio sine qua non" for the nonspecific effect. In the case of insulin and

its action from the skin deposit, the involuntary nervous system may play a similar important part, and like experimental conditions might allow us to prove it. The involuntary nervous system is, in general, divided into two different parts, the sympathetic and the parasympathetic.

If one wished to interrupt the parasympathetic part by pharmacodynamic substances, two methods are feasible. The parasympathetic part can be blocked by increasing the sympathetic tonus by adrenalin, but this method is not suitable for our experiments. As mentioned above this would lead to an immediate increase of the blood-sugar level and thus hide the insulin effect, and one could not determine if the effect is eliminated by interrupting the parasympathetic system or by an overpowering glycemia following the adrenalin injection.

The other possibility is to paralyze the parasympathetic system by atropine. Atropine, if given in doses of 10 mg. per kg. body weight, does not affect the blood sugar. Therefore it was possible to use the blocking action of atropine for our studies. We injected a series of animals with the same amount (0.5 unit) of insulin per kg. body weight employing the intradermal, the subdermal and the intravenous mode of administration in the same animals after a period of rest of at least seven days. Simultaneously with the injection of insulin, atropine was injected subcutaneously, and, in some experiments, this atropine injection was followed by another injection of the same dosage thirty minutes later. Blood-sugar determinations were made at intervals of thirty minutes, and the following results, tabulated below, have been found:

TABLE I

THE EFFECT OF ATROPINE ON THE ACTION OF INSULIN, AS MEASURED BY THE CHANGE IN BLOOD-SUGAR CONTENT (PER CENT)

RABBIT	INTRAVENOUS		SUBCUTANEOUS		INTRADERMAL	
	30 MIN.	60 MIN.	30 MIN.	60 MIN.	30 MIN.	60 MIN.
87	–59.0	–44.8	–36.2*	–56.7	–15.3*	–45.3
88	–54.5	–43.9	–53.8*	–59.7	–21.5*	–35.6
76	–58.5	–52.5	–44.7*	–65.8	–18.8*	–58.6
58	No experiments		–45.6	–49.7	–27.8	–47.3
78	–49.5	–22.0	–33.4*	–47.8	–10.4*	–32.0
79	–47.4	–10.0	–47.3	–49.1	+ 6.9	+13.8
	–61.9	–40.5				
82	–35.9	+17.9	–16.0*	–28.0	+ 4.6*	+ 5.3
Average	–52.4	–36.93	–39.57	–50.97	– 9.25	–25.4

*A second injection of atropine at the end of thirty minutes following the first bleeding.

TABLE II

THE CHANGE IN BLOOD-SUGAR CONTENT AFTER INJECTION OF INSULIN ALONE (PER CENT)

RABBIT	INTRAVENOUS		SUBCUTANEOUS		INTRADERMAL	
	30 MIN.	60 MIN.	30 MIN.	60 MIN.	30 MIN.	60 MIN.
87	–53.2	–34.0	–47.7	–50.8	–34.4	–46.6
88	–46.3	–19.0	–55.9	–57.3	–27.9	–44.2
76	–62.9	–33.1	–34.1	–52.1	–19.8	–44.7
58	No experiments		–47.3	–50.3	–19.7	–31.2
78	–55.7	–33.9	–39.3	–44.4	–19.9	–36.8
					–26.5	–48.5
					– 7.4	– 8.3
79	–47.0	–0.0	–24.8	–40.7	–12.5	–40.0
82	–12.5	+23.2	–30.3	–26.8	– 1.6	+ 4.1
Average	–46.2	–16.1	–39.91	–46.06	–18.5	–32.9

If one compares these findings with the control results without atropine, the difference is striking. With atropine the intradermal injection of insulin is much less powerful than when the same animal is injected with insulin alone.

Furthermore, the effect of the intradermal injection does not reach the same level and is much less effective than after subdermal injection. If one compares both series of subdermal injections, with and without atropine, in some of the animals, atropine has lessened the effect of the drug a little, but in most cases not at all.

Comparing the intravenous injection and its effect, there is no difference whether or not atropine has been added. Thus it is shown that atropine, which does eliminate to a certain degree the action of the parasympathetic nerves

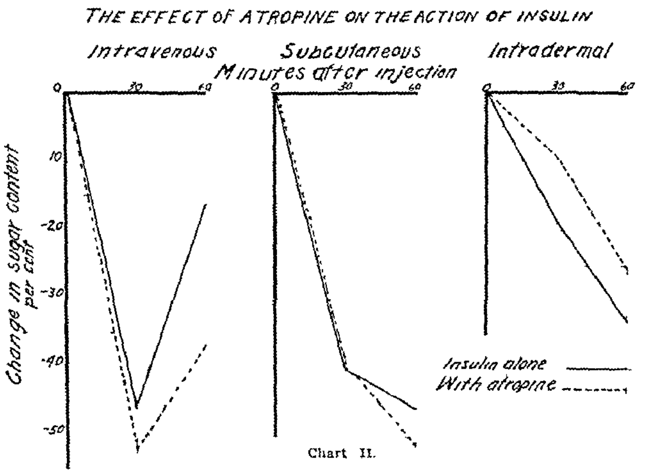

Chart II.

for a certain time, changes the action of insulin according to the method of administration. The intravenous and the subdermal injections of insulin are not affected at all, while the intradermal injection loses one-half of its power. This shows without doubt that atropine, which probably does not undergo any chemical combination with insulin but which without doubt paralyzes the function of the parasympathetic system to a certain degree, has diminished the insulin action. *This diminution is possible only if the paralyzed parasympathetic system has played a part in the insulin action.*

We have previously stated that the direct hormone action within the circulation and within the body fluids cannot be the only basis of the blood-sugar lowering effect. *We have stated that there must exist another route of insulin* action outside the circulatory system, and this other action must be especially powerful by intradermal injection, less by subdermal, and almost lacking by intravenous administration. The atropine experiments show that by blocking the parasympathetic system, a great deal of the insulin action is lost after intradermal injection, none is lost after subdermal and none after intravenous

administration. *This demonstrates that the missing link in the explanation of the large insulin effect from an intradermal deposit is represented by an action of the involuntary nervous system.* We cannot conclude from these experiments directly in what way the involuntary nervous system is able to produce this blood-sugar lowering effect, but we know, from other experiments in human beings and from the physiologic experience of blood-sugar metabolism, that the liver, to a certain degree, is responsible for the blood-sugar balance by forming glycogen and glucose, respectively. We have learned from experiments initiated by Claude Bernard, that stimulation of the sympathetic system results in a glucose formation. It is further known that the stimulation of the parasympathetic system results in a glycogen formation.

Minkowski's[6] experiments have proved that not only depancreatization but also the severing of the nerves between pancreas, duodenum and liver results in complete diabetes, thus showing how much the nerves participate in the pancreatic and hepatic action. It would take much too long to enter into details provided by the extensive research done on diabetes in almost every country. But from the facts mentioned we know that parasympathetic action leads to an increase of the glycogen formation, and we feel justified in concluding that the definitely proved part that the involuntary nervous system plays in insulin action consists of a nervous stimulation of the glycogenic function of the liver.

This explains how insulin, even if present only in small amounts of the active form in the tissues, may create a pronounced effect upon the blood-sugar level. The stimulus, traveling along parasympathetic fibers from the skin to the liver can result very quickly even though insulin is not present in the blood stream, in a glycogenetic process which takes glucose from the circulation, thus diminishing the blood-sugar level. This also explains how it is possible that the total effect of the same amount of insulin is less after subdermal than after intradermal injection. After subdermal injection the drug enters the circulation comparatively quickly and acts there as a hormone. It loses its power by being eliminated from the blood stream. In intravenous injection the action depends upon the dosage, i.e., upon the actual amount of insulin present. From the combined experiments with intravenous insulin injection and atropine administration we know that practically no neural effect exists if insulin is present only in the blood stream.

We are not able to say to what extent the subdermal tissues provide a neural stimulus as compared with the skin; but we know that the nerve effect from the skin is the most intensive, and, as the deposit is being diminished, the nerve action is lost. (We have to consider that it is impossible to eliminate the entire nerve action. If this were possible it would probably result in a complete loss of all insulin effect so long as only a deposit existed in the skin and no insulin were absorbed into the circulation.) This would account for the early onset of the actual blood-sugar lowering effect from the skin deposit. It also accounts for the more lasting effect and explains the reason for such a large total effect after intradermal injection. Small amounts of insulin will continue to be absorbed from the intradermal deposit after the entire amount of insulin, which has been injected subcutaneously, has been exhausted. Smaller

amounts may still enter the blood stream from the intradermal deposit, while the above-mentioned nerve effect is still present. This makes it possible that, even after the exhaustion of the subdermally- and intravenously-given insulin, the blood-sugar reducing effect may bring the total amount of actually metabolized sugar to a higher degree than any other kind of insulin administration.

It may be mentioned in this paper that experiments in human beings with the same object in view have shown similar differences following intradermal and subdermal injection, thus confirming these findings.[7]

Many other theoretic conclusions may be drawn from the above statements, and, in connection with the many publications on insulin, it may be possible now to explain several observations on the basis of this nerve effect. Magenta and Biazotti[8] first showed that atropine has an antagonistic action to insulin. Many other authors point to similar ideas. Giving more details would lead beyond the limits of our paper. We merely wanted to show that many previous publications have pointed in the same general direction, but we believe that the difficulty in determining more exact data has been that the entire previous work was based only upon one kind of insulin administration, namely, the subdermal injection. The observed differences between the intradermal and the subdermal administration gave us the clue to develop the reasons for this difference.

CONCLUSIONS

Our conclusions are: A deposit of insulin in the body acts by a nerve stimulation, very probably of the glycogen-forming function of the liver. This nerve stimulation is conducted by way of parasympathetic fibers. The effect is active as long as the insulin deposit exists. It is stronger if the insulin deposit is made in an organ which has an especially close relationship to the involuntary nervous system, as has the skin, and if the absorption is very slow. It is weaker after deposition of the insulin in organs where absorption takes place rapidly, for instance, the subdermal tissues, and is lacking after intravenous administration. The neural effect of insulin does not depend upon the dosage of the deposit, while the hormone action in the circulation and in the body fluids does depend upon the dose. Simultaneously with the beginning of the absorption from the insulin deposit and with the entrance of insulin into the circulation, the direct hormone effect is manifested. With this hormone effect and the conclusive destruction of the hormone and the elimination by natural channels, the nerve effect decreases, together with the diminution of the insulin deposit outside of the circulation, and disappears as the last part of the insulin deposit is absorbed.

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THE EFFECT OF ORALLY ADMINISTERED MUCIC ACID UPON RENAL FUNCTION*

By William C. Rose, Ph.D., and Richard W. Jackson, M.S., Urbana, Ill.

In a series of investigations on the relation of chemical structure to the nephropathic action of the dicarboxylic acids we[1] have shown that mucic acid, when administered subcutaneously as its sodium salt, is extremely destructive to the kidneys. The injection of 0.25 gm. to 0.50 gm. in rabbits induces a severe nephritis of the tubular type, accompanied by an almost complete or total loss of the power of phenolsulphonephthalein elimination, and an enormous retention of nitrogenous waste products in the blood. In some cases anuria occurs, and persists for a period of one to two days following the acid administration.

In the course of our discussion of these results, we pointed out that the data may have a practical value, inasmuch as it is reported that mucic acid is now being manufactured on a large scale with a view to its use as a component of baking powders and other food products. Obviously, the fact that mucic acid exerts a deleterious action when introduced parenterally, does not justify one in assuming that the same effect would follow its oral administration. It is quite conceivable that this substance, like some other dicarboxylic acids when given per os, may be rejected by the alimentary cells, and thus fail to come in contact with the kidneys. Furthermore, should the acid be absorbed, there is the additional possibility that it might be detoxicated by the organism, and in this fashion be prevented from exerting injurious renal effects. With these considerations in mind we have conducted a series of experiments involving the feeding of mucic acid.

Rabbits were employed as the experimental animals. In all cases food was withheld, but water was supplied *ad libitum*. This procedure was followed in order to insure constant conditions throughout the experiments, inasmuch as animals refuse to eat after the development of severe nephritis. Furthermore, Salant and his associates[2] have shown that certain foods render rabbits less susceptible to the action of tartaric acid. It seemed safer, therefore, to eliminate the possibility of dietary influences by using the animals in a fasting condition. Results so obtained doubtless may be regarded as maximum effects.

As criteria of renal function, we have determined at frequent intervals the quantities of nonprotein nitrogen, creatinine, and chlorides in the blood. After control periods of approximately forty-eight hours, during which time one or two blood samples were removed, the animals received the mucic acid by stomach sound either in the free state, or after neutralization with sodium carbonate. The administration of the sodium salt almost invariably evoked a marked diarrhea, probably due to the liberation in the stomach of relatively

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large amounts of sodium chloride. For this reason, in most of the experiments, the acid was suspended in water and given in the free state. For details as to the methods of analysis, the reader is referred to a former communication.[3]

Twelve experiments were made upon twelve different animals. The doses of mucic acid varied from 1 to 19 gm. With the smaller doses (1 to 5 gm.) no effects upon renal activity were observed. With the larger doses (5 to 19 gm.) nephritis was occasionally seen. Indeed, four of the twelve animals manifested well-defined cases of tubular involvement. The results of two positive experiments are shown in Tables I and II. In the first, two 5 gm. doses of mucic acid were administered on succeeding days. As will be observed, the nonprotein nitrogen of the blood increased from approximately 42 mg. per hundred cubic centimeters (a normal value for rabbits) to 74 mg. per hundred cubic centimeters. The blood creatinine rose from 1.7 to 4.4 mg. per hundred cubic centimeters, while the chlorides remained practically constant.

In Table II are presented the results of an experiment in which 19 gm. of mucic acid were given during a single day. The data indicate enormous increases in both nonprotein nitrogen and creatinine. On the fourth day following the acid administration, the nonprotein nitrogen amounted to 228 mg. and the creatinine to 17 mg. per hundred cubic centimeters of blood. These are the most pronounced retentions we have observed in any of the animals following the oral administration of mucic acid. In a third experiment, the

TABLE I

RABBIT NO. 2, 2230 GM.

DATE	MUCIC ACID FED.	BLOOD ANALYSIS			NOTES
		N. P. N.	PREF. CREATININE	NaCl	
Nov.	gm.	mg.	mg.	%	
19					No food; water ad lib.
20		42.1	1.7	—	8:30 A.M.—10 c.c. blood removed.
21		42.3	1.7	0.51	8:30 A.M.—10 c.c. blood removed.
21	5.0				11:00 A.M.—Acid given as sodium salt.
21		43.5	2.0	0.51	8:30 P.M.— 9 c.c. blood removed.
22		62.5	3.4	0.55	1:30 P.M.—10 c.c. blood removed.
22	5.0				2:30 P.M.—Acid given as sodium salt.
22		74.3	4.4	0.52	10:30 P.M.—10 c.c. blood removed. Animal was unable to stand, and was chloroformed.

TABLE II

RABBIT NO. 4, 2570 GM.

DATE	MUCIC ACID FED.	BLOOD ANALYSIS			NOTES
		N. P. N.	PREF. CREATININE	NaCl	
Jan.	gm.	mg.	mg.	%	
9					No food; water ad lib.
10		42.6	1.7	0.51	8:15 A.M.—10 c.c. blood removed.
11		41.7	1.7	0.51	8:15 A.M.—10 c.c. blood removed.
11	19.0				*Free acid given in two equal doses at 10:50* A.M. and 1:30 P.M.
12		58.8	2.8	0.54	8:30 A.M.—10 c.c. blood removed.
13		151.6	8.7	0.52	8:30 A.M.—10 c.c. blood removed.
14		219.6	14.0	0.54	9:30 A.M.—10 c.c. blood removed.
15		228.8	17.0	0.56	9:00 A.M.—10 c.c. blood removed. Experiment discontinued.

results of which are not shown in detail, the feeding of 15 gm. of the acid led to values for nonprotein nitrogen of approximately 200 mg.

It is evident from the above data that in rabbits the administration of mucic acid by the alimentary route *may* cause nephritis, but that the dose necessary for such an effect is enormously larger than that which destroys kidney function when the substance is injected subcutaneously. Similar results in the case of tartaric acid have been observed by others. Pearce and Ringer[4] obtained indications of nephritis in one of the three animals in which tartrates were administered per os. Post[5] was unable to observe in man any detrimental effects upon the kidneys following the administration of medicinal doses of Rochelle salts. Apparently mucic acid, like tartaric acid, is largely unabsorbed from the alimentary tract. Only overwhelming doses, such as were administered in some of our experiments, lead to renal involvement in normal animals.

The above data may be interpreted by some as indicating the harmlessness of mucic acid in the quantities which would be present in baking powders. On the other hand, we believe that such an interpretation would be unfortunate. Although the doses of mucic acid employed in our experiments are infinitely larger than could be obtained in man from the quantities of baking powders ordinarily ingested, it is not impossible that the habitual ingestion over long periods of time of minute traces of the substance *might* lead eventually to renal effects in some individuals. Furthermore, we know comparatively little concerning the mechanism of alimentary absorption, and still less regarding variations which may occur in the permeability of the alimentary cells under abnormal conditions. While the "selectivity" of the cells might effectually prevent mucic acid absorption under favorable circumstances, who knows but that increased permeability in abnormal conditions might permit the passage into the circulation of injurious traces of the substance? Inasmuch as nontoxic acid compounds are available for use in baking powders, it would not appear rational to employ a violently nephropathic agent, and depend upon its rejection by the gastrointestinal cells. Therefore, we seriously question the advisability of the use of mucic acid as a component of the human dietary.

SUMMARY

1. Of twelve rabbits which received doses of 1 to 19 gm. of mucic acid by mouth, four showed distinct evidence of renal involvement, as indicated by marked retention of nitrogenous waste products in the blood.

2. These results would seem to indicate that mucic acid is scarcely a safe substance to be employed as a component of human food products.

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THE VALUE AND CHARACTER OF REPORTS AND RECORDS OF TISSUE EXAMINATIONS*

By L. A. Turley, A.M., Ph.D., Oklahoma City, Okla.

THE value of tissue examinations is so well recognized that we need waste little time discussing this point. The question before us is the nature of the report and the manner of keeping the records of these tissue examinations. At the meeting of this Society a year ago, the statement was made in an informal lecture that all the surgeon wanted to know was whether or not the tissue examined revealed malignancy. This statement was a surprise to me. I can scarcely believe this is true, but, if it is, it is squarely up to us, as pathologists, to educate the surgeons to a value of tissue examinations beyond this point. Any surgeon who is satisfied with the meager report of "positive" or "negative" in regard to the question of malignancy is fair neither to himself nor to his patients, because he is not availing himself of the information as to the character of the lesion with which he is dealing, and, consequently, he is robbing the patient of the proper treatment. As an example of this, I might briefly recite a recent case in our hospital. The patient came in with symptoms of intestinal obstruction accompanied by loss of weight. Physical examination revealed a mass in the right flank. A diagnosis of malignancy was made from the findings and operative procedures were carried out on the basis of this diagnosis. The postoperative gross diagnosis confirmed the preoperative diagnosis, but the microscopic examination showed the lesion to be tuberculous, which, of course, entirely changed the prognosis and after-treatment of the case. If the surgeon had proceeded with a simple negative report of nonmalignancy and had been satisfied with this report, he would have been utterly in the dark as to the condition with which he was dealing.

There is no laboratory report carried on at the present time which simply indicates positive or negative in regard to any one specific question where any other report is possible. Blood counts are not reported on the basis of positive or negative leucocytosis or erythrocytosis, but a detailed report is made of the exact number, not only of any individual hemocyte but of all of them. In urine examinations, physicians are no longer satisfied with positive or negative reports of the various substances which might be encountered; but, if positive, they desire to know the degree of excess. Similarly in blood chemical procedures, not only a knowledge of the presence of the normal and abnormal but the amount of normal and abnormal constituents is desired. So that we may sum up the proposition as to the character of the report of the laboratory procedure by saying that laboratory reports should be both qualitative and quantitative, for often the amount of abnormality is as important to know as

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the presence of the abnormality. The same thing applies to tissue reports. As was stated before this body last year and as has recently been stated by Ewing, even in cases of positive malignancy, not only the fact of malignancy, but the character of the malignancy should be reported, for it is well recognized that different malignancies differ in degree and habits so that not only immediate treatment but after-treatment and prognosis depend on knowing the character of the malignancy. And, further, tissue examination, whether malignant or not, should be reported in detail. In case of inflammations, the definite type of inflammatory process should be reported, as acute, chronic, proliferative, destructive, catarrhal, diphtheritic. Nor should we stop with some one word, such as those enumerated above, which might characterize the particular inflammation. The pathologist should record in detail all pathologic conditions found, and these should be described in such a way that the diagnosis is apparent to any one who understands pathology at all. In this way and in this way only is the clinician furnished with valuable information for the treating of the present lesion, and also with data by the comparison of which, in a series of cases with the clinical findings, he is enabled in the future to make more accurate diagnoses and differentiations. From my experiences in examining tissues, there has arisen in my mind the question, "Is it not true that appendicitis and salpingitis are confused, because the examination of the salpinx, which was the primary subject of operative procedure, often shows far less disease than the appendix, which was removed as a matter of routine during the operation?" There are also cases in which appendicitis and gastric ulcers have been confused, and it is only by means of the reports of the pathologist that the clinician is furnished with the data necessary to distinguish between these pathologic conditions. Therefore, if the clinical pathologist, in the examination of tissues, wishes to render the service of which he is capable, to the clinician and to the patients, it is necessary that he go into considerable detail, describing the pathologic conditions of all of the tissues in the particular specimens submitted and including the findings in his report to the clinician.

We now come to the consideration of the character of the record of tissue examinations which should be kept. Let us, in this consideration, divide the record into two classes: material and documentary. The material records consist of the slide or slides from which the examination was made. This necessitates the use of a technic which would produce a permanent mount in preparing the slides for diagnosis. In general routine tissue examinations, the time element of the diagnosis is not so imperative but that some of the slower procedures which develop permanent mounts may be used. In suspicious cases, the quicker frozen method, of course, must be employed. But in these cases, if the rapid technic does not give a permanent mount, a permanent mount should be made, as nearly as possible, from the same site as the rapid preparation. The slide, once made, should be carefully labeled and filed. Slide cabinets, with a capacity for thousands of slides, take up very little room. The preservation of the slide is necessary for many reasons: The progress of the case may take such a turn that the clinician would like a review of the examination. On occasion, the clinician may desire confirmation of the opinion of the pathologist by some other pathologist, a request with which, of course, he should

freely and cheerfully comply. The discrepancies in diagnosis, which frequently occur in such cases, are often due to the fact that the two men are examining different slides. In a bone tumor recently submitted to me, three different diagnoses could have been made, each equally correct, depending on the portion of the lesion from which the slide was made. Further, the diagnosis may be questioned and the pathologist himself is protected by having on hand the slide from which he made his diagnosis. Such slides are also valuable as subjects of study in case of research, and, for other minor reasons, the slides which have been examined should be carefully preserved.

The other type of record mentioned above is called documentary for the reason that it is written out and should consist of a report either made to the clinician or placed on the chart of the patient for consideration by the clinician. This report should include a careful and detailed description of the microscopic examination including all pathologic conditions found on examination. It should include the details which would, for example, differentiate the diagnosis of scirrhous carcinoma from medullary carcinoma; the size and general condition of the fibroblasts in a spindle-cell sarcoma, the proportion and distribution of stroma in regard to the tumor cells, etc. In the prostate gland, for example, the atrophy or hypertrophy of the tissues composing the organ; the increase or decrease in stroma; the amount, character, and distribution of infiltrating cells, the presence or absence of edema or congestion; the condition of blood vessel walls, etc., all should be carefully made out and recorded. When such a description is complete, the clinician will be able to know the exact state and condition of the afflicted organ, and the diagnosis will be evident.

In our own hospital we proceed as follows: First, there is a description made of the gross material brought from the operating room or the autopsy table, and in all cases this should be a description, not a diagnosis. For example, "blood vessels were injected," should be used instead of the word "congestion." This description can be written by anyone, even the laboratory technician, and sometimes it is best to have the description written by some one in this capacity, because there is too much of a tendency on the part of medically trained people to diagnose instead of describe. The number, size, general shape, appearance and differences in appearance of the different parts of the gross material are included in this description. This serves as a means of comparison between the record of the operation and any tissue examination that may be made, and makes certain that both are talking about the same material, for it often happens that those who have charge of the tissues in the operating room get the tissues from different cases mixed. If the laboratory has the gross description of the material that is accredited to a given case, however, all such mistakes can be corrected before diagnosis, so there is no chance of confusion later. Second, the part of the gross specimen which should be examined microscopically is determined by directions from the surgeon or interne or by the pathologist. Third, when the slides are made and ready for examination, the section is inspected in the gross by transmitted and refracted light and is described in the gross, giving size, shape, staining reactions, apparent arrangement of tissue and other points that can be made out by the naked eye. Then follows the description of the slide as seen through

the microscope, followed by the diagnosis. Fourth, a copy of this description is kept by the laboratory and a copy attached to the patient's chart or sent to the clinician if it is an outside case.

There may have arisen in your minds a question as to the value of all this and a conclusion that there is a lot of labor entailed that is not justified by the results. If such is the case, I beg to differ with you. From time to time through this paper I have indicated some of the values of this type of a tissue report. These may be summarized as follows: first, it insures an examination of the right tissues in each case. The appearance of these tissues in the gross is a valuable thing to the clinicians, internes and others. By comparison of this gross description with the microscopic description later on, all concerned become better pathologists and better clinicians. The accurate description of the microscopic examination not only gives in a word or two a diagnosis of the most important pathologic findings, but reveals all of the pathology present, and will ultimately enable the pathologist to form more accurate and differential conclusions with regard to certain pathologic conditions. These conditions might be grouped under a class, as, for example, the habits of growth of tumors, the differences in different inflammations of the same organs and parts. By a study of these reports the pathologist is continually training himself to be more accurate, and hence is able to make more valuable diagnoses. It gives the clinician the fullest possible picture of the processes with which he is dealing. By recording the accurate details from which the diagnosis is made the pathologist is protecting himself in case his diagnosis is questioned. A collection of such pathologic reports enables the clinician at the end of the year to review his cases and by comparing the pathology with the clinical findings is enabled to make more accurate diagnoses in the future. And, last but not least, it is building an invaluable library for research work. Any one interested in any type of pathology would be enabled not only to review the data in a given hospital but also to secure from other hospitals reports and material from similar cases, and so have available a large field of examples from which to draw pathologic conclusions. In this way, and in this way only the pathologist renders the present generation, not only in his own immediate vicinity, but throughout the country, a valuable service. He also renders future generations an invaluable service when he collects material which can be used in clarifying and making definite all pathologic queries and concepts.

DISCUSSION

Dr. Robert A. Keilty.—Frozen sections were mentioned in the paper. For the past five years we have made all our routine surgical pathologic sections, averaging about three a day, by the frozen method. We have almost discarded the paraffin method. We make the section on the first day or, preferably, after twenty-four hours fixation in 4 per cent formalin. I put in a plea for this method; the gross appearance is fresh in mind, the report is quickly gotten out of the way, and the clinical service receives the final diagnosis while the case is uppermost in their minds—not days or even weeks later.

Dr. Ralph G. Stillman.—Gross examination of specimens, which is the only record for the laboratory of a specimen as it comes from the operating room, should be made with greatest possible care. The gross appearance of specimens rapidly changes, and the appearance after it is fixed is quite different from that of the fresh material. It is important

that gross descriptions should be passed upon by the older man in the laboratory. The majority of our sections are filed in the form of frozen sections. We do make many paraffin sections, but we have no difficulty in getting good frozen ones. Sixty-five or seventy-five per cent of our specimens are reported on, either on the same day or the next day. We have no trouble with the frozen sections in permanent mounts.

Dr. A. H. Schade.—I would also like to make a plea for frozen sections. We use frozen section work routinely. We have sections that run back many years; for instance, we have one section that we have had for thirty years. Recently we have been doing quite a bit of work with tissue the size of a pinhead. We mount them in 10 per cent gelatin, freeze and cut, stain with hematoxylin and eosin, and they give good detail. If the frozen section work is done properly, you will get all the data you wish for microscopic diagnosis.

Dr. James B. Bullitt.—I would like to learn how a man can make a frozen section the size of a pinhead with any satisfaction. You can do so in paraffin sections very readily. In the matter of time too, I think a great deal of emphasis has been put upon the time in making a paraffin section. Frozen sections are, of course, very much quicker. After the specimen has been fixed a few hours with formaldehyde it does not take over seven or eight hours to finish the section by the paraffin method. The slower method gives better results.

Dr. Frank W. Hartman.—I would like to emphasize the relative merits of fixed frozen sections and paraffin or celloidin sections with regard to the pinhead-sized pieces of tissue that have been mentioned. It is difficult certainly to make both types of sections from such a small bit of tissue, and, if the frozen section does not give the desired results, usually there is not enough left to make paraffin sections. I do not believe that it is necessary to adhere to either method for all types of work. We use paraffin routinely for autopsy work and fixed frozen sections usually in the surgical work. Occasionally, however, we would like very much to get a second section from a particular area in the surgical work, and with a paraffin block we could do this to best advantage. In addition, the paraffin blocks can be filed, serving as a safeguard in case the tissues or slides are lost.

Dr. Leon S. Lippincott.—I want to say just one word more in favor of frozen sections. We do not have any trouble. We have made it a routine to save our tissues for at least a month, and we throw away only those of no interest. We have no trouble with pinhead pieces of tissue.

Dr. L. A. Turley (closing).—I wish to thank the men for the discussion of this paper. There are a number of interesting things that have been brought up in the discussion of frozen and paraffin methods, which is a subject in itself. The main difficulty with frozen work is that it is particularly difficult to get a section without loss of cells. This is especially true unless the specimen has already been fixed or if there is very much edema, or if the tissue is very cellular. You do not get a complete picture. On the other hand, the paraffin method so contracts the tissues, and sometimes ruins them if the reaction of the fixative is not right, that the picture is distorted. You can be sure that all the cells are contracted, some more than others. I have had no difficulty with pinhead specimens. With reference to the point raised about the gross description: I do not mean by that that just any one could come in and make the description, but it is not necessary to have a medically trained man, in fact it is better not to have one so trained for reasons pointed out in the paper. By saving these specimens you are collecting valuable material for research.

COCAINE-EPINEPHRIN MIXTURE, ITS TOXICITY AND ANTIDOTE*

By Ellison L. Ross, Ph.D., M.D., Chicago, Ill.

MAYER[1] states, "Ross reported that epinephrin greatly increases the toxicity of cocaine for cats by intravenous injection, basing his conclusion almost entirely on the amounts required to stop the respiration. Ross' conclusions throw little light on the therapeutic use of epinephrin with cocaine and the committee wishes to state that epinephrin in very small amounts does not increase the toxicity of procaine when injected into the subcutaneous tissue or about the nerve." Later in the same article it is stated that, "whether in doses of one milligram or less is capable of increasing the toxic action of cocaine appreciably is not known and this question is in great need of investigation." There are gross errors in these statements made by Mayer. First Ross[2, 3, 4] did not base his conclusions "almost entirely on the amounts required to stop respiration," but also upon changes in the blood pressure, the sensations, and visible reactions of people given the drugs. Second, Ross[2] did not state that epinephrin in very small amounts increased the toxicity of procaine, but gave positive evidence to the contrary. Third, Ross[3] has shown the effect of epinephrin in amounts less than one milligram. Adrenalin (1:1000), as stated in the article, was used in the solutions of cocaine in a concentration from 0.2 to 10 per cent. Calculating from the data given in Table V, it is found that epinephrin was used in doses varying from 0.012 to 0.0250 milligrams per kilogram of body weight. Table A contains the calculated amounts of epinephrin given, in addition to the essential data in the original article.

Table A

PER CENT ADRENALIN*	WITH COCAINE	VOL. SOL. INJECTED PER KG.	MG. EPINEPHRIN PER KG.	MG. COCAINE PER KG.
0.20	¼	6.0 c.c.	0.012	15.3
0.40	¼	5.6 c.c.	0.032	14.1
1.00	¼	4.1 c.c.	0.041	10.3
5.00	¼	3.1 c.c.	0.156	7.7
10.00	¼	2.5 c.c.	0.250	6.3

*Adrenalin is 1:1000 as stated in paper, or 1/10 per cent epinephrin.

In view of the decided toxicity of cocaine with adrenalin, an effective treatment should be available. For simple cocaine poisoning Bastedo[5] recommends ice bag to head and whiskey or large doses of bromides. Underhill[6] states that for depression and collapse from cocaine, stimulants such as caffein, aromatic spirits of ammonia, or hot alcoholic drinks are to be given. Sollman[7] directs the use of aromatic spirits of ammonia and caffein, and says that "amyl nitrite has been recommended." Paulson[8] says of acute cocaine

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poisoning that slight attacks of syncope yield to the assumption of a horizontal position, and if there is great pallor, inhalation of amyl nitrite should be tried. Haines[9] states that inhalations of amyl nitrite and hypodermic injections of nitroglycerin are "signally successful." Cushny[10] states that for acute cocaine poisoning amyl nitrite has been advised when the blood pressure seems much elevated.

Since the observations of the author in previous work have noted the extreme changes in blood pressure associated with the corresponding changes in the minimal lethal dose, it was thought well to investigate the action of atropine with the mixture of the two drugs. With the high blood pressure produced by cocaine and adrenalin there was always a decided stimulation of the vagus With the high pressure and increased period of cardiac relaxation, conditions would be ideal for the development of an acute and fatal cardiac dilatation. Atropine checks the action of the vagus and so might possibly prevent this. Only three animals were necessary to convince us of the worthlessness of atropine as an antidote for the mixture of cocaine and adrenalin. The results are given in Table I.

TABLE I

EFFECT OF ATROPINE ON M. F. D. OF COCAINE-ADRENALIN

CAT NO.	% COCAINE	% ADRENALIN	% ATROPINE	M. F. D. GRAMS OF COCAINE HCl PER KG. WT.
1	¼	5 of 1:1000	.01–½ c.c. per kg.	0.0041
2	¼	" " "	" " " " "	0.0063
3	¼	" " "	" " " " "	0.0060
Average	¼	5 of 1:1000	.01–½ c.c. per kg.	0.0055

Experiments were next made with the nitrites, as they are the most effective for reducing blood pressure and are the drugs recommended by several authorities[7, 8, 9, 10] as antidotes for cocaine alone. Two series of dogs were observed The first series was given ½ c.c. of 25 per cent solution of chloretone in olive oil per kilogram, intraperitoneally. The animals were then put upon the operating board and given enough ether to allow the attachment of the proper apparatus for making the injections and measuring the blood pressure. The ether was removed, and, after ten minutes' observation, injections with the Woodyatt machine of ¼ per cent cocaine hydrochloride and 5 per cent adrenalin (1:1000) was begun. When respiration stopped the animals were considered to have received the lethal dose. The second series of animals was treated just the same as the first except that after the normal blood pressure had been taken 1/20 c.c. of spiritus glycerilis nitratis was injected intraperitoneally. Tables II and III give the results obtained.

From Table II we find that the average minimal lethal dose of the cocaine and adrenalin mixture for nine dogs was 0.012 grams of cocaine hydrochloride per kilogram of body weight. This indicates that the mixture is not quite as toxic for dogs as for cats. The M.F.D. determined in a previous paper for cats was 0.0077 grams. The average change in blood pressure was enormous, being from 85 to 235 millimeters of mercury.

From Table III, it is found that the addition of nitroglycerin had in-

TABLE II

M. F. D. AND BLOOD PRESSURE FROM COCAINE AND ADRENALIN WITHOUT NITROGLYCERIN

DOG NO.	% COCAINE	% ADRENALIN	M. F. D. GM. COC. HCl PER KG.	B. P. BEGINNING	B. P. MAX.
1	¼	5 of 1:1000	0.015	80	268
2	¼	" " "	0.011	70	196
3	¼	" " "	0.010	110	244
4	¼	" " "	0.015	64	244
5	¼	" " "	0.012	108	204
6	¼	" " "	0.010	90	210
7	¼	" " "	0.014	170	298
8	¼	" " "	0.011	80	238
9	¼	" " "	0.012	90	212
			0.012	85	235

TABLE III

M. F. D. AND BLOOD PRESSURE FROM COCAINE AND ADRENALIN WITH NITROGLYCERIN

DOG NO.	% COCAINE	% ADRENALIN	DOSE NITROGLYC.	M. F. D. COC. PER KG.	B. P. BEGINNING	B. P. MAXIMUM
1	¼	5 of 1:1000	1/20 c.c. per kg. Sp. Nitrog.	0.012	110	216
2	¼	" " "	"	0.023	120	212
3	¼	" " "	"	0.021	170	270
4	¼	" " "	"	0.014	128	244
5	¼	" " "	"	0.024	146	234
6	¼	" " "	"	0.021	72	206
7	¼	" " "	"	0.019	82	210
				0.019	118	227

creased the minimal lethal dose over 50 per cent. The change in blood pressure was still very great, being from 118 to 227. Apparently nitroglycerin did not prevent the development of the maximal blood pressure of which the system was capable.

From a comparison of the results in these two tables it must be concluded that nitroglycerin does not in these doses prevent the development of great rises in blood pressure and that nitroglycerin does reduce the toxicity of cocaine-adrenalin for dogs.

To establish the fact that nitroglycerin is effective in reducing the toxicity of a mixture of cocaine and adrenalin completely, further work was thought necessary. A series of 10 cats was treated in the same manner as the series of dogs, except that ¼ c.c. of a 0.2 per cent suspension of nitroglycerin per kg. was injected in place of the smaller dose and half as much chloretone was given. The results are embodied in Table IV.

The average M.F.D. of cocaine with adrenalin was found to be 0.0147 grams of cocaine hydrochloride per kg. of body weight. Without nitroglycerin the M.F.D. is 0.0077 grams of cocaine hydrochloride per kg. This means that cocaine and adrenalin so administered are approximately half as toxic with nitroglycerin as without it.

All of the work thus far on this problem had been done on animals anesthetized with chloretone while the cocaine-adrenalin mixture was injected intravenously with the Woodyatt pump. It was proposed to give cocaine and adrenalin intraperitoneally to normal cats, to some with nitroglycerin, and to some without it. A series of 5 cats was given 1 c.c. of 3 per cent cocaine

TABLE IV

EFFECT OF NITROGLYCERIN ON M. F. D. OF COCAINE-ADRENALIN

CAT NO.	% COCAINE	% ADRENALIN	DOSE NITROGLYCERIN	M. F. D. GRAMS COCAINE HCl PER KG. BODY WT.
1	¼	5 of 1:1000	¼ c.c. of 2% per kg.	0.021
2	"	" " "	" " " " " "	0.012
3	"	" " "	" " " " " "	0.017
4	"	" " "	" " " " " "	0.013
5	"	" " "	" " " " " "	0.015
6	"	" " "	" " " " " "	0.011
7	"	" " "	" " " " " "	0.013
8	"	" " "	" " " " " "	0.017
9	"	" " "	" " " " " "	0.012
10	"	" " "	" " " " " "	0.016
Average	¼	5 of 1:1000	¼ c.c. of 2% per Kg.	0.0147

TABLE V

EFFECTS OF A FATAL DOSE OF COCAINE AND ADRENALIN

CAT. NO.	PER KG. DOSE DRUG	SALIVATION	TWITCHINGS AND SPASTICITY	CONVULSIONS	DEATH
1	1 c.c. Coc. HCl 3% and Adren. 30% 1:1000	10 min.	27 min.	80 min.	2 hr. 20 min. chloroformed after 4 severe convulsions.
2	" " " " "	45 min.	60 min.	1 hr. 45 min.	Recovered. Severe vomiting at 2 hr. 15 min.
3	" " " " "	80 min.	2 hr.	None	Chloroformed. Much prostrated.
4	" " " " "	20 min.	20 min.	123 min.	Found dead 8 hr. after dose.
5	" " " " "	30 min.	50 min.	None in 1st 3 hr.	Found dead 8 hr. after dose.
6	" " " with Nitroglycerin	70 min.	None	None	Recovered
7	" " " "	None	3 hr. 10 min.	None	Recovered
8	" " " "	None	None	None	Recovered
9	" " " "	None	None	None	Recovered

hydrochloride and 30 per cent adrenalin (1:1000) per kg. of body weight. Three died promptly in convulsions; one developed such a hopeless condition that it was chloroformed, and one recovered. The animals trembled and became severely salivated and spastic. Dilatation of the pupils occurred in each case after the first several minutes Then incoordination of movements developed. Convulsions were brought on by an attempt to move about the cage. The convulsion was always severely clonic throughout the body.

Another series of 4 cats was given the same dose of cocaine and adrenalin just after an intraperitoneal injection of $\frac{1}{10}$ c.c. of spiritus glycerylis nitratis per kg. of body weight All of these cats recovered. One cat developed some salivation, and another developed some spasticity and twitching. The results are tabulated in Table V. It is clear from the results tabulated here that nitroglycerin prevents death of cats given the usual lethal dose of cocaine and adrenalin.

CONCLUSIONS

1. Atropine did not reduce the toxicity of cocaine with epinephrin.
2. Nitroglycerin did not reduce in dogs the rise in blood pressure due to injections of cocaine with epinephrin.

3. Nitroglycerin reduced the toxicity about 30 per cent for dogs in the doses used and under the conditions of the tests.

4. Nitroglycerin administered to cats under anesthesia reduced the toxicity of a cocaine-epinephrin mixture approximately 50 per cent.

5. Fatal doses of cocaine-epinephrin for normal cats can be made nonfatal by the administration of nitroglycerin.

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THE USE OF SODIUM THIOSULPHATE IN DIAGNOSTIC PROCEDURE*

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THE continued use of sodium thiosulphate is confirming all of the early desires of those who predicted its efficacy in the treatment of the metallic toxemias. About a year ago it was used successfully on several cases of lead poisoning following the absorption of lead tetraethyl. The use of sodium thiosulphate in many refineries and on workmen employed at lead smelteries has again indicated its value. Reports from these industrial heads show that its efficacy is satisfactory. The continued application of the drug in arsenic intoxication shows it to be the most satisfactory means of combating this disturbance, and as a result the application of syphilis therapy has been greatly extended. It has been shown by us that the simultaneous use of the drug with the arsenical does not affect the therapeutic activity of salvarsan. There are several reports from abroad showing that sodium thiosulphate may be used simultaneously with neosalvarsan without producing any change in the therapeutic activity.

In previous articles it has been shown that arsphenamine produces a direct action upon the involuntary nervous system.[1] This has been shown by localization of the arsenic, and by leucocyte counts made upon the blood at various intervals. Alteration in the extent of this intoxication may be brought about by means of sodium chloride, glucose, lactose, and a variety of other means, such as changing the balance of the constituents in the blood stream and the tissues.

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Nyiri[2] has developed the use of sodium thiosulphate as a kidney function test. These investigations have shown that when the drug is introduced into the blood stream, 60 to 70 per cent of it is oxidized to sodium sulphate, while the remaining 30 to 40 per cent remains unchanged in the urine, provided there is a normal kidney function. In abnormal kidney function these values fall between 1 and 23 per cent. The technic employed consists in the use of 10 c.c. of a 10 per cent solution injected intravenously after the bladder has been emptied. The urine is then collected every hour for three hours. The thiosulphate excreted is determined by iodine titration. Nyiri states that as a result of 120 experiments the test is practicable, requires small expenditure of time, makes small demand on the patient, and is quantitative. The injections are painless and safe. Eskelund[3] also employed the test in 31 cases with good results and advocates the continuation of the method.

From the diagnostic point of view little attention has been given to the use of sodium thiosulphate. It has been pointed out by one of us[4] that in an intoxication by a heavy metal, there is a comparatively small amount of that metal present in the blood stream or in the urine. This is particularly the case in jaundice, dermatitis, dermatitis exfoliativa, and scleroderma, showing a pigmentation, and the ordinary leather-like development of the skin. In practically every case of this nature, the blood stream and urine may show a low value, except in a few cases where saturation has developed. Under this condition a higher value may be expected in the blood and in the urine.

These patients may develop the intoxication either through the therapeutic application of arsenicals or through the therapeutic application of remedies highly contaminated with arsenic, or through the ingestion of foods containing glucose, soluble cocoa, apples, potatoes, tomatoes, etc. The contamination in this last group is brought about through chemical processes or as a result of the use of insecticides containing arsenic. The possibilities of infant food supplements are also being considered as a cause of scleroderma observed in several children.

The fact that arsenic is an intoxicant to the autonomic nervous system makes it probable that the cellular activity has been modified to such an extent that there is a deposition of the drug with a resulting modification of the properties of a normal skin. The dermatologist readily diagnoses these cases, but is always at a loss as to the proper procedure from a therapeutic point of view.

For diagnostic purposes specimens of blood and urine should be obtained before any injections are made. In order to secure the best results the daily intravenous administration of 0.5 gram of sodium thiosulphate is advisable. A twenty-four-hour specimen of urine is obtained and this is analyzed for arsenic. An occasional specimen of blood is obtained and checked up for the change in arsenic values. An increase of arsenic in the blood and urine is followed by clinical improvement. In most instances a steady increase of arsenic output is noted. This is followed by gradually diminishing quantities until a uniform output is obtained. Under these conditions the pigmentation decreases, the skin gradually becoming normal in texture and feeling.

Under some circumstances the drug in crystal form may be administered by mouth. In treating children, 0.3 gram is given before meals, three times each day. Adults receive one gram three times each day preceding the meal. Intestinal irritation is the only contraindication to the use of the drug. In many instances it behaves as a cathartic.

Only four patients will be used for the purpose of illustrating the many cases that fall into this class. In the first instance, an adult woman illustrates the diagnostic value of the drug. The second is an adult man with the diagnosis of arsenical pigmentation. The third case is that of a child, about four and one-half years old, representing scleroderma with pigmentation. The fourth is a young girl with arsenical pigmentation. The patients are designated as *A*, *B*, *C* and *D* in the tables showing the total solids and the rate of arsenic excretion.

PATIENT *A*.—Diagnosis of scleroderma was made with the origin unknown. Past history of patient showed that she had been feeling normal in every respect. The first symptoms consisted in the feeling of tightness of the skin over both knees, followed by the same condition in the region of the ankles. The lumbar regions next showed involvement with strips of parchment-like zones extending downward and around the body to the abdomen. The neck, wrists, hands and feet became involved. The feeling of the skin was very similar to parchment and the patient was unable to move the hands and fingers readily. Thyroid treatment was given with no results. General condition of the patient was good. Duration from first onset about one year. Examination of the urine showed arsenic *trace* and the blood 0.03. Treatment with sodium thiosulphate by mouth was instituted, giving 1 gram daily. Urine showed the presence of 1.54 mg. of arsenic per 100 grams of dried specimen on the second day. At the end of thirty days, the excretion was 0.60 mg. of arsenic, and at the end of six months' time the value had reached the normal excretion rate in which 0.11 mg. of arsenic was found. The patient was discharged from the hospital with physical condition good and the skin normal in every respect. Further questioning brought out the fact that the patient worked with a furrier and that the absorption had taken place as a result of handling the furs which had been sprayed with arsenic. At the present time it is reported that the patient is well.

PATIENT *A*

SPECIMEN	DATE	TOTAL SOLIDS	ARSENIC IN MG. PER 100 GM. OF DRIED SPECIMEN
Blood before treatment	11/ 9/23	22.50	0.03
Urine before treatment	11/ 9/23	2.54	Trace
Urine	11/10/23	2.18	0.535
Urine	11/11/23	2.05	1.540
Urine	11/12/23	1.77	1.150
Urine	11/13/23	2.13	0.552
Urine	11/14/23	2.09	0.611
Urine	12/16/23	1.71	0.696
Urine	12/17/23	1.53	3.921
Urine	12/18/23	1.91	0.496
Urine	12/19/23	2.33	0.141
Blood	12/19/23	24.70	0.049
Urine	1/15/24	2.61	0.230
Urine	1/16/24	2.61	0.240
Urine	1/17/24	2.54	0.213
Blood	1/18/24	18.28	0.140
Urine	3/20/24	2.74	0.110

PATIENT *B*.—Roumanian; age, thirty-nine. Twenty-four years in the United States; occupation: telegrapher. Onset: four years age a group of "pimples" appeared in the left scapular region accompanied by intense itching and burning. The rash spread over this region and involved the labium, the trunk, the anterior aspect of both thighs, both forearms, hands and feet. The rash was characterized by grouped vesicles on an erythematous base. Intermingled with the fresh lesions were scars and pigmentation. The clinical diagnosis was dermatitis herpetiformis.

Two years ago patient began taking arsenic in the form of Asiatic pills, averaging 9 pills a day for four months. He then camo to the hospital Dec. 20, 1923, remaining until March, 1924. During that time he received Fowler's solution up to 18 drops a day, and his condition improved steadily. He continued taking Fowler's solution for two months after leaving the hospital, then went back to private physician who put him on Asiatic pills and a tonic. He took 200 of the pills in the next two months. During this time his skin became steadily darker until he was pigmented from head to foot with the exception of the flexor surfaces of the great joints, the axillae, the anterior surface of the elbows, groins and popliteal spaces. In addition to the above-mentioned pigmentation, there was a moist eczematoid dermatitis of the face, ears, neck and genital-anal region. The pigmented areas on the trunk and limbs were covered with small follicular miliary papules which at times showed vesicular formation on the summit. At the back of the neck the skin showed a papulomatous condition; the palms showed keratoses. Clinical diagnosis of an arsenical dermatitis in addition to Duhring's disease was made.

He returned to the hospital in September, 1924, and was immediately given sodium thiosulphate intravenously, 1 gram every other day, receiving a total of 45 injections. In the meantime he had taken 1 gram of the thiosulphate crystals by mouth three times a day after meals. The pigmentation faded gradually and the patient's general condition was much improved. Neurologic tests: negative. Lymph nodes generally enlarged.

The present condition of the patient is satisfactory.

PATIENT *B*

SPECIMEN	DATE	TOTAL SOLIDS	ARSENIC IN MG. PER 100 GM. OF DRIED SPECIMEN
Urine	9/25/24	2.64	0.10
Urine	9/26/24	2.28	0.48
Blood	9/26/24	21.34	Trace
Urine	9/27/24	1.52	2.19
Urine	9/29/24	3.18	2.63
Urine	10/ 2/24	1.60	0.36
Urine	10/ 4/24	1.73	0.34
Urine	10/ 6/24	2.12	Trace
Urine	10/ 8/24	1.80	0.17
Urine	10/10/24	2.33	0.61
Urine	10/16/24	3.96	0.12
Urine	10/17/24	2.99	0.19
Urine	10/18/24	3.97	1.27
Urine	10/21/24	2.75	0.05
Urine	10/23/24	4.66	0.05
Urine	10/25/24	2.37	0
Urine	10/27/24	5.26	0
Urine	10/31/24	3.09	Trace
Urine	11/13/24	4.44	0
Urine	11/15/24	3.44	0.16
Urine	12/ 2/24	5.38	0.08
Urine	12/ 4/24	4.99	0.08
Urine	12/ 6/24	4.52	2.29
Urine	12/ 8/24	5.30	1.90
Urine	2/19/25	4.27	0.34

PATIENT *C.*—Jewish, age, four. Patient has suffered for several years with itching and scleroderma. Patches of pigmentation are found on the neck, the trunk, and the buttocks. These areas of pigmentation closely follow nerve distribution. Sodium thiosulphate was administered by mouth in doses of 0.3 grams three times a day before meals. Patient is still under observation. Marked improvement in pigmented areas is noted.

PATIENT *C*

SPECIMEN	DATE	TOTAL SOLIDS	ARSENIC IN MG. PER 100 GM. OF DRIED SPECIMEN
Urine	12/30/25	1.61	Trace
Urine	12/31/25	2.97	0.157
Urine	1/ 1/26	3.02	Trace
Urine	1/ 2/26	3.02	0.138
Urine	1/ 3/26	1.62	0.452

PATIENT *D.*—Italian, female, age, eleven. The patient has suffered since infancy with a lichenified papular eruption most marked on limbs but involving trunk and face. Clinical diagnosis: Prurigo hebra.

On January 12, 1925, examination showed the pigmentation with the same description as in case *C.* The same follicular lichenified papules were also present.

Injections of sodium thiosulphate: Marked improvement is noted in the lichenified pigmented eruptions. Patient very markedly improved. Still evidence of prurigo. Last visit 3/18/25.

PATIENT *D*

SPECIMEN	DATE	TOTAL SOLIDS	ARSENIC IN MG. PER 100 GM. OF DRIED SPECIMEN
Blood	1/ 5/25	22.76	0.04
Urine	1/ 5/25	3.23	0.08
Urine	1/29/25	2.09	Trace
Urine	3/ 2/25	5.04	0.087

From the data given above it is apparent that the use of sodium thiosulphate has distinct advantages in confirming some doubtful diagnoses and the etiologic factors involved. A general principle that has been observed in such conditions as described above is that the *blood* and *urine* usually show a value for arsenic that is either zero, trace, or a quantity below the normal. During the past year Myers and Cornwall showed that arsenic is a normal constituent of the body and the embryo as well.

These clinical symptoms as illustrated in the cases above are frequently observed but the etiology has been obscure, the treatment unsatisfactory. As a result of studies in blood chemistry which will be published in another article, the use of sodium thiosulphate has been demonstrated to be satisfactory. Solutions of thiosulphate deteriorate and lose much of their therapeutic activity and for this reason it has been found advantageous to use solutions prepared from sterile crystals. These solutions give the best results. The toxicity of sodium thiosulphate is approximately 6,000 mg. per kilogram of body weight. On this basis it is readily recognized that it is difficult to imagine a condition in which an undue toxicity could arise. In spite of this low toxicity it has been found that doses of 0.5 gram either daily or every other day give more satisfactory results. Large doses produce a

too rapid exosmosis which is followed by an exacerbation. Injections are continued until permanent relief is obtained.

It has been pointed out in earlier publications that arsenic of inorganic origin is more difficult to remove than that which is deposited from the anti-syphilitic remedies.

SUMMARY

Sodium thiosulphate in its active form is useful in the diagnosis of scleroderma in which arsenic is a contributing factor. It is employed in such cases as those in which a pigmentation is observed. Continued use of fresh crystals of sodium thiosulphate releases the arsenic that has been deposited either in the skin, or along the nerve trunks until a gradual return of normal conditions is observed. The parchment-like conditions of the skin disappear under treatment and the pigmented areas disappear. A branny desquamation very frequently accompanies clinical improvement. Thus far no evidences of kidney dysfunction have been observed. In fact, quite the reverse is noticed, namely a stimulation of diuresis. The ingestion of large volumes of fluid is recommended. The patients respond promply to treatment and the clinical symptoms improve.

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THE PREPARATION OF SODIUM MORRHUATE*

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SODIUM morrhuate is regarded by many as practically a specific in the treatment of tuberculosis, particularly in so-called "surgical tuberculosis." The substance is not at present available on the American market and must be prepared as needed. In view of certain difficulties which I have experienced in its preparation and the encouraging results which I have obtained with a good product in the treatment of several cases of tuberculosis coming under my care within the last few months, it has occurred to me that lack of a proper supply of the drug may be responsible in considerable measure for the backwardness of the profession generally in establishing the therapeutic value of this interesting substance. Accordingly, the purpose of this communication is to record some observations on the preparation and physical characteristics of the drug, a knowledge of which is an essential preliminary to successful clinical experimentation with it, though I must reserve for a future discussion such clinical data as I myself have been able to collect.

Sodium morrhuate, when properly prepared, is a somewhat bulky, distinctly yellow powder, with a characteristic piscine odor and a greasy, soapy feel. It dissolves readily in water and produces a solution which is light yellow in color, clear as crystal, and which forms a lather, like other soaps, when agitated. Though little emphasis has been placed on the fact by others who have written on the subject, sodium morrhuate is a relatively unstable substance when preserved under ordinary conditions; in solution it changes little in appearance even when kept in untinted glass and exposed freely to the atmosphere, except that, at the end of a few days or a week, a fine, cloudy, white precipitate settles to the bottom of the container. When clinically tested, the solution does not remain therapeutically active much longer than two weeks even when kept in amber bottles, sterile, and tightly sealed, distinct deterioration of the drug being apparent often within four or five days. The powdered substance not in solution turns somewhat darker on standing and, if not kept in a desiccator, tends to become resinous in consistency. The powder seems to deteriorate more rapidly than the solution; at least at the end of a few days it no longer goes completely into solution as formerly, but forms a murky precipitate, however much care may have been taken in the interim to keep it dry and protected from the light.

Because of the difficulty experienced in ridding the substance of water in the final stage of its preparation so as to obtain a fine dry powder, it first seemed reasonable to assume that the keeping qualities of the drug might be enhanced by placing and storing it in a desiccator, away from contact with all moisture, but apparently the substance deteriorates more rapidly under these

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conditions than when preserved in the ordinary way without taking any special precautions. This is also in accord with the observation, previously noted, that watery solutions of the drug do not deteriorate with undue rapidity, even though they are comparatively dilute (3 per cent).

Accordingly, it seems probable that oxidation is the important factor in deterioration, and it would therefore seem necessary to protect the drug by all feasible means from oxidation both during and after preparation. This end may be accomplished by simplifying and expediting the preparation of the drug, on the one hand, and by final preservation in an oxygen-free medium, on the other.

The technic for the preparation of sodium morrhuate was devised by Sir Leonard Rogers, but he has nowhere recorded, nor has anyone else so far as I am aware, a sufficiently detailed account of the process to enable one not particularly adept in chemical procedures to prepare the substance without considerable experimentation. A more or less complete and detailed account of the process, as I have used it, follows:

Twenty gm. of sodium hydroxide are weighed out with rough accuracy and are dissolved in water to make 150 c.c.; 200 c.c. of 95 per cent ethyl alcohol is added to 100 gm. of cod-liver oil in a 500 c.c. Erlenmeyer flask, and the sodium hydroxide is added, a little at a time, with shaking.

A layer of clear yellow oil now floats on a somewhat darker layer of water, alcohol, and sodium hydroxide. The flask is heated to the boiling point under a reflux condenser, care being taken to avoid bumping, and is kept boiling until saponification of the cod-liver oil is complete, i.e., until the supernatant layer of oil, which grows smaller and smaller the longer the solution boils, entirely disappears, and the contents of the flask assumes a dark amber color; two hours usually suffices for this, though Rogers advocates boiling twice as long.

Twenty per cent sulphuric acid is now added slowly from a burette until a turbid oily precipitate just begins to form and settle to the bottom of the flask; the excess of alkalinity is thus neutralized. Boiling is now continued in the absence of the reflux condenser until most of the alcohol has been evaporated. If carried too far, the solution becomes concentrated, and the flask cracks. At this stage, the solution is thick, dark amber in color, and clear; it is now mixed in a fairly large evaporating dish with 200 gm. of dry, coarse-grained sand, the latter having been previously washed with hydrochloric acid and then with water to neutrality.

The mass is placed on a water-bath and evaporated to dryness as quickly as possible; this takes several hours at best. When dry the plastic sandy mass is placed, a portion at a time, in an extraction thimble and extracted with ether in Soxhlet's apparatus. The ethereal extract is dark amber in color; it should be clear except for a colorless, gelatinous, stringy precipitate which appears when moisture is present in the substance extracted, the precipitate representing water-soluble substances which come through the thimble only to precipitate out again in the ether of the flask. This precipitate really does no harm because it is removed during washing incident to the next procedure. The ethereal extract is next poured into a fairly large separatory funnel, and an excess of 20 per cent sulphuric acid is added to liberate the fatty acids; the amount

needed is variable, but the presence of an excess acidity is readily determined by allowing a drop to fall from the bottom of the separatory funnel onto a strip of litmus paper. The fatty acids are now thoroughly washed with from three to five portions of distilled water until the wash water is no longer acid to litmus paper; this is readily accomplished by allowing the fatty acids to settle out in a distinct layer on top of the wash water and then draining away the latter. The fatty acids during this process appear as a clear oily solution only in case sufficient ether is present to keep them completely dissolved; otherwise they tend to separate out as a solid.

The washed fatty acids are now delivered into a medium-sized evaporating dish, 100 c.c. of alcohol is added, followed by a saturated solution of sodium hydroxide, a drop at a time, to the point of exact neutrality to phenolphthalein. The latter, if added directly to the solution, gives the final product a dark yellow or light brown color; both theoretically and practically this appears to do no harm, though the presence of this substance in the sodium morrhuate is not particularly to be desired; furthermore, the use of an indicator is really unnecessary, since during the process of neutralization the solution itself undergoes a delicate change of color at the end point. Even with the use of phenolphthalein it is not necessary, of course, to add the substance directly to the solution, a drop-reaction plate being at hand.

The evaporating dish is now placed on the water-bath, and the soap evaporated to dryness, the sides of the dish being scraped occasionally with a spatula. This drying takes an hour or more, and, even though th heating process be continued for several hours, it usually becomes necessary to place the product in a desiccator overnight before it can be properly powdered with mortar and pestle. Prolonged heating on the water-bath and prolonged drying in the desiccator must be avoided, both causing very perceptible darkening of the product; a preparation which may be virtually white in a semi-dry condition may rapidly become darker in color as a result of excessive heating, and at the end of twelve hours may have become so dark a brown as to render it presumably unfit for use.

In outline, then, the above process consists in the saponification of cod-liver oil in the presence of alcohol by means of sodium hydroxide and heat, neutralization of the excess of hydroxide, extraction with ether, separation of the fatty acids with an excess of acid, purification by washing with water, and, lastly, resaponification with sodium hydroxide to the point of neutrality.

While theoretically simple and technically by no means difficult, this method yields results which are not uniform. The fatty acids entering into the final product are mainly unsaturated, and very little is known concerning their physical or chemical properties; presumably they occur in different relative and absolute proportions and amounts in different samples of oil, though this presumption hardly seems to account for all the difficulties sometimes experienced in preparing a first-class product.

Reference has already been made to the instability of sodium morrhuate under ordinary conditions, but I have discovered that if it be sealed in a glass tube under a vacuum it keeps much better, since by this method oxygen is excluded. This may readily be accomplished by weighing out convenient

amounts of the substance into test tubes of small diameter, heating the glass tube a suitable distance above the upper level of the powder, drawing out the tube quite thin, attaching a Chapman pump, creating as complete a vacuum as possible, and then applying the point of a flame to the most constricted part of the tube to complete a seal.

Since it seems almost certain that prolonged heating during any part of the above-described process interferes with the efficacy of the drug, and since that portion of the technic which calls for drying after mixing with sand entails by far the longest heating, any modification of the process which would do away with this step would appear to be highly desirable. A convenient means for accomplishing this is to "salt out" the soap by saturating the solution first obtained as a result of saponification (after neutralizing the excess of hydroxide) with sodium chloride; the soap is thus formed immediately as a sticky, semisolid mass from which the excess of water is readily expressed. complete drying on the water-bath being readily accomplished. The crystalline sodium chloride serves as an excellent substitute for sand, so that the ether extraction is accomplished without delay. This modification I regard as highly valuable, and I now use it in preference to that previously described. The sodium chloride is insoluble in ether and remains behind in the extraction thimble of the Soxhlet, and such small amounts as might find their way through the thimble are, of course, subsequently removed during the process of washing the fatty acids.

For clinical use sodium morrhuate is dissolved in distilled water in 3 per cent solution and placed in ampules or vaccine bottles and sterilized; tricresol may or may not be added. Injections are best made intravenously, starting with 0.1 to 0.3 c.c. and increasing by 0.1 c.c. with each dose to a maximum of about 1.0 c.c.; injections are given at intervals of from three to five days. Fresh solutions should be prepared at least as often as once a week.

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INABILITY TO ESTABLISH THYROID TOLERANCE IN WHITE RATS*

By J. Alexander Clarke, Jr., M.D., Philadelphia, Pa.

CARLSON, Rooks and McKie[1] in early experiments on thyroid intoxication in various animals mention the possibility of establishing a tolerance to thyroid extract. The present experiment was started to obtain some information about such a tolerance if existant. None could be demonstrated as a result of thyroid finding, but, rather, a condition of hypersensitiveness appeared to be developed.

Thirty-two young rats of about 100 grams, the offspring of tumor-bearing rats, were selected. Of these twenty were males and twelve females. They were kept in two cages, males and females being separated. The diet was to consist, in addition to the thyroid, in unlimited bread and milk. This diet was the same as that used for the tumor-bearing rats in adjoining cages, which rats were well and healthy except those in the last stages of large tumor growth. It was soon found that this diet was insufficient for the thyroid fed rats, due, presumably to the greatly increased appetites of these rats. Before this condition was realized and remedied, five had died, two controls and three experimental rats. It may also have hastened the death of some of the experimental rats later in the experiment.

The thyroid used was commercial, dried extract in powdered form from two reliable companies.† The doses were weighed individually on an analytical balance and mixed with bread crumbs and water to form a paste. Feeding was generally easy, especially after the thyroid had been administered a few days. The appetite soon became voracious and continued so until death. One death occurred within a half hour after eating. There were two exceptions to this and in both cases the experiments had to be stopped because the rats refused to eat more thyroid.

The symptoms noted were the same as those of other observers. The earliest was an increase in the appetite. The fur became rough and the rat became thin without much loss of weight. This thinness was particularly apparent along the spinal column, which became very prominent. Shortly before death the rats became restless, often developed a diarrhea, lost weight rapidly, became too weak to stand, and died. If one waits for a pronounced loss in weight as an index to toxemia, the rat will usually die. Recovery after cessation of thyroid feeding is remarkably quick, the rat usually gaining 10 to 20 gm. in a week; the diarrhea subsides rapidly and the rat becomes normal in appearance. The increase in the susceptibility to infection was also noted. There were infections in the thyroid rats and none in the controls.

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†The iodine content of these two powders was, unfortunately, not obtained. The change was made on March 21, and, since there is no decided change in the curves at this date, we assume they were of approximately the same strength. There were four deaths from the first and six from the second powder.

Three methods were tried to raise the tolerance. First, to see if one attack of toxemia provided any protection against a second poisoning. Second, to start with a dose that was tolerated and to increase this dose gradually. Third, to give doses that were tolerated at intervals of one week for a long period before starting the toxic doses.

EXPERIMENT 1.—Starting Feb. 9 five rats were fed a concededly toxic dose of 0.5 gm. daily. On Feb. 24 one rat had diarrhea and had lost 25 gm. Feeding of this rat was then stopped. On March 1 another rat died after losing 35 gm. in weight. Of the other three rats, on March 1, one was at the original weight while the other two had lost 15 and 20 gm. each. Feeding of all rats was then stopped until March 11. At this time all four rats appeared healthy and had gained considerable weight (smallest 25 and largest 35 gm.). Thyroid feeding was then resumed, 0.5 gm. daily. On March 22 the rat which showed the first symptoms of intoxication died after a loss of 30 gm. His first feeding lasted fifteen days and he still lived, the second feeding lasted about eleven days and ended in death. On March 29 two rats died after a loss of 10 and 35 gm., respectively. This

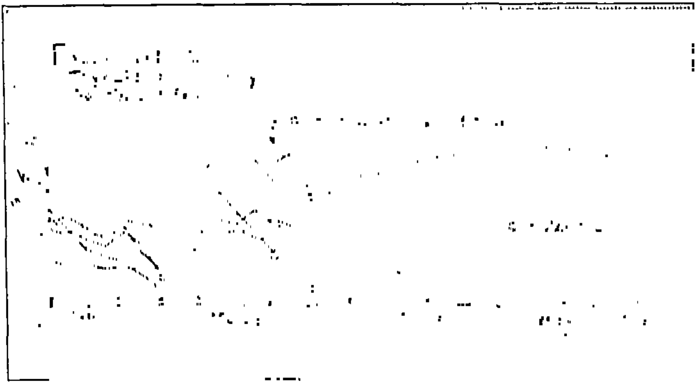

Chart I.

feeding lasted eighteen days, while the first lasted twenty-one days The feeding of the remaining rat was stopped, but the feeding had been so difficult during the last week it is doubtful whether this last week should be considered a part of the experiment. There was a gain of about 10 gm. in weight during the period. When last seen on June 4 the rat weighed 150 gm. and seemed none the worse for two thyroid intoxications.

EXPERIMENT 2 was similar to experiment 1 except that 0.1 gm. of thyroid was fed daily instead of 0 5 gm. Feeding was started on Feb. 9 in all rats. On Feb. 21 and Feb. 23, respectively, two rats died and another rat showed such a great loss of weight that thyroid feeding was stopped on Feb. 18. Since it was during this period that one of the control rats died, it is probable that these deaths were either due wholly to the feeding deficiencies mentioned above, or were largely influenced by them. In any event they should not be considered thyroid deaths. The rat whose feeding was stopped on Feb. 18 gained 35 gm. in weight and was again fed daily doses of 0.1 gm. from March 1 until he died, April 5. Three days before death there had been only a loss of about 7 gm., although other evidences of thyroid intoxication were present, notably weakness. This 145 gm. rat took thyroid extract, 0.1 gm. for 35 consecutive days, before succumbing. This record is only surpassed by one other rat, and this was a 200 gm. rat.

Of the remaining rats in this experiment, one died on March 1 after being fed 0.1 gm. since Feb. 9, with a loss of 25 gm. Feeding was stopped on the other rat on March 1, during which period there had been a loss of 20 gm. At this time the rat was not considered in a dangerous condition. On March 11, after a gain in weight of about 18 gm., feeding was again started at 0.1 gm. daily. On March 30 the rat was very weak, had diarrhea, and, although there was a gain of almost 10 gm. in weight for the period of feeding, the feeding was stopped. Recovery was rapid (a gain of 20 gm. in the first week), and, when last seen on June 4, was alive and healthy, having gained 75 gm. This rat then had two thyroid feedings with an interval of nine days between. The first lasted twenty-one days without alarming symptoms, while the second lasted twenty days and left the rat in a dangerous condition.

EXPERIMENT 3 was designed to start at a dose below a toxic dose and endeavor to show tolerance by gradually increasing the dosage. Accordingly, the five rats were fed 0.01 gm. thyroid daily, this being approximately 1/10,000 of their body weight.

Of the five rats used in this experiment, one was so very obviously abnormal that it should not be considered in the experiment. This rat weighed 10 gm. less on June 4 than

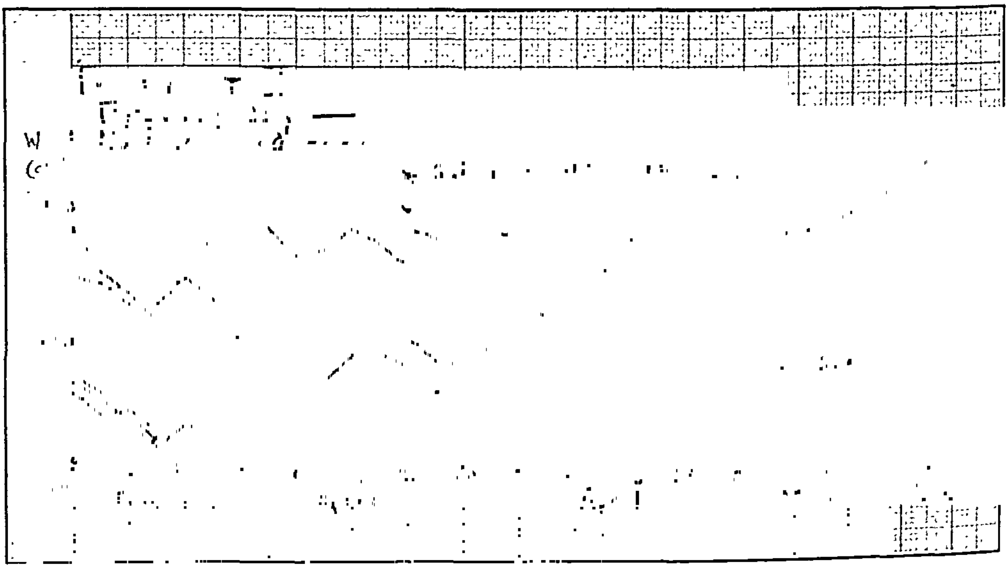

Chart II.

it did at the start of the experiment on Feb. 9. In this rat 0.01 gm. of the thyroid caused a loss of weight of 30 gm. in twenty-eight days. For seven days 0.02 gm. was fed, when the rat became very weak and had diarrhea. After this there was only a 20 gm. gain in weight, ten of which was lost. For some unknown reason this rat either was more than ordinarily susceptible to thyroid or the synchronism of the alarming symptoms with the thyroid feeding was a coincidence.

The other rats were all fed on the same schedule, to wit: 0.01 gm. daily for fifty-three consecutive days, Feb. 9 until March 23; 0.02 gm. for seventeen days, March 23 to April 6; 0.04 gm. for seven days, April 6 to April 13; 0.07 gm. for seventeen days, April 13 to April 30.

During the first period all rats thrived, showed no symptoms of thyroid poisoning, and there was a gain in weight, 30, 28, 8 and 5 gm., respectively. During the same period, the five surviving control rats gained 65, 38, 20, 20, 8 gm., respectively, which about proves that 0.01 gm. is a harmless dose for rats.

During the second period the rats gained as follows (same order as above) 20, 0, 8, 0 gm. During the third period, 0.04 gm. daily, there was no gain in weight nor was there any loss.

During the last period, 0.07 gm. daily, one rat died April 25, showing no lose in weight nor any of the symptoms of thyroid intoxication. The last weight recorded was the death weight. The three remaining rats all showed a loss of weight, 15, 15, and 20 gm., respectively, although none had diarrhea. Feeding was then stopped and was immediately followed by a very rapid gain in weight, at least 20 gm. in the first week, which is very fair proof that all were suffering from too much thyroid. When last seen on June 4 they had gained 60, 40 and 50 gm., respectively.

This experiment offers more hope of establishing tolerance than either of the two preceding ones. It is possible that the definite loss of weight without the diarrhea noticed in the other rats may have been an evidence of tolerance to these symptoms. Had the increase been more gradual the results might have been different.

EXPERIMENT 4 was originally planned to produce a case of thyroid intoxication by large, single, weekly doses and then to see if this intoxication would prevent further intoxication with the same doses. Accordingly 5 rats were fed 1.0 gm. in one dose once a week. In some instances it was necessary to take two days to the feeding, but in every

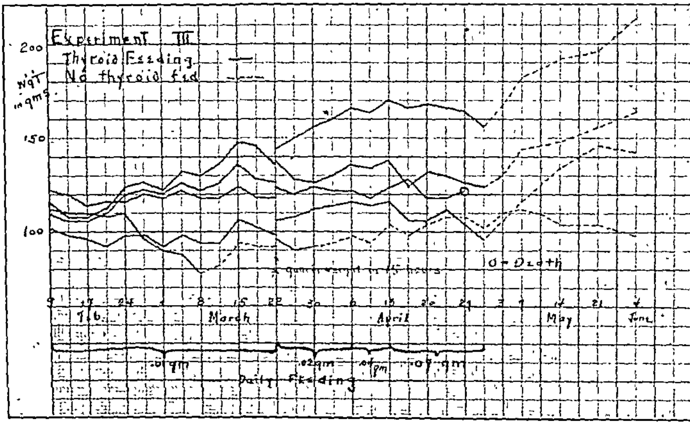

Chart III.

case the full gram was given in not less than two consecutive days. We were greatly surprised to find that all five rats thrived and gained weight on 1.0 gm. in a single dose when 0.7 gm. weekly in seven doses consistently produced alarming symptoms or death. The dose was then divided and fed at intervals of less than a week, keeping the total weekly dose approximately the same. When 0.3 gm. every other day was reached, the symptoms of intoxication appeared in all rats.

There were three periods of feeding, the first 1.0 gm. weekly from Feb. 9 to March 30, 50 days; the second from March 30 to April 20, 21 days; and the third from April 20 to April 30, 10 days.

During the early part of the first period the gain in weight was not great, and in two rats there was some loss. The rather rapid gain during the last part of this period may be an evidence of tolerance, but, since the feeding conditions were also improved at this time, the evidence is far from conclusive. The gains during the whole period were as follows: 50, 43, 23, 15, and 15 gm. During the second period 0.5 gm. was fed twice a week, and the weights remained about stationary. During the third period all rats lost weight except one, the losses being 15, 13, 10, 7 gm. One rat absolutely refused to eat any more

thyroid and feeding was discontinued on April 27. Within a week this rat had gained over 30 gm. Of the remaining four rats three made the usual rapid recovery immediately upon the withdrawal of the thyroid, although the actual weights were not so great, chiefly because of the fact that these rats had already attained their full growth when the intoxication began. One rat, however, did not gain upon the withdrawal of the thyroid and died April 7 with bloody diarrhea, one week after stopping the thyroid. This was the only case in which recovery did not occur if the rat lived twenty-four hours after the last dose.

Experiment 5.—In this experiment 0.1 gm. was fed once a week from Feb. 9 to March 23, 45 days, and then 0.1 gm. daily until symptoms of intoxication appeared. One rat died Feb. 23, probably because of the early feeding difficulties mentioned above. During the first, the period of weekly feeding, the rats gained as follows: 60, 50, 20 and 10 gm., respectively. After 14 days of 0.1 gm. of thyroid daily the two rats which had made the small gains were very weak and the feeding was discontinued. Both of these rats made

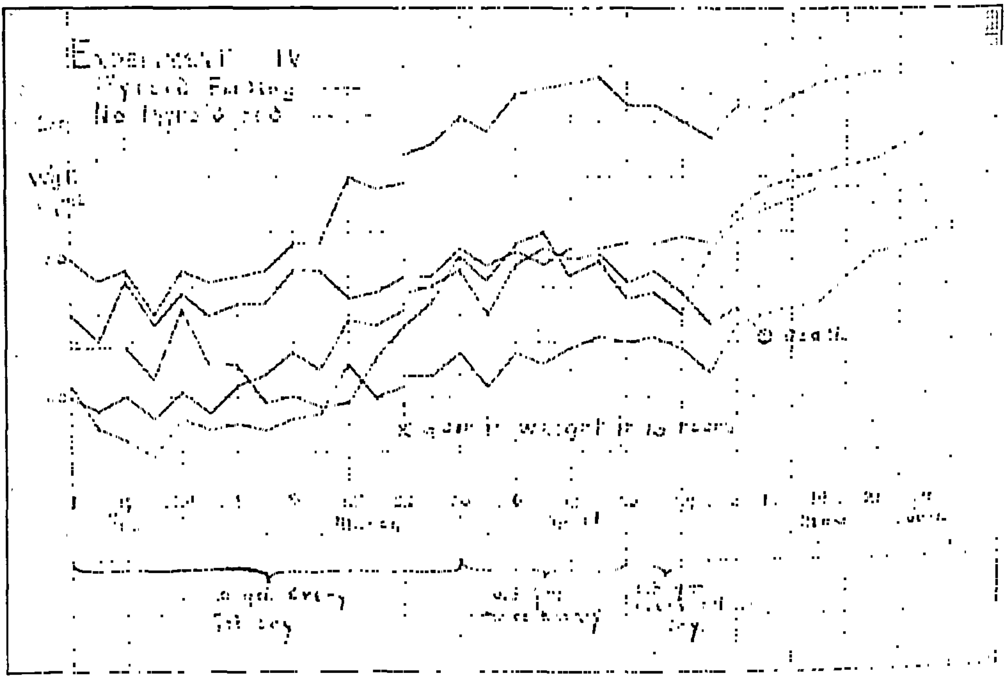

Chart IV.

the usual quick recovery and subsequently became normal healthy rats. There was very little loss in weight in the two remaining rats, about 8 and 12 gm. One rat took 0.1 gm. daily until April 16, 24 days, at which time it had lost 30 gm. in weight and was very weak. The remaining rat took 0.1 gm. daily until April 30, when the feeding was stopped, a period of thirty-eight consecutive days. At this time the rat had lost but 20 gm. in weight and was not considered in a serious condition. There was the usual rapid gain, however, 20 gm., during the first week after withdrawal. This was the longest consecutive period of feeding and may be accounted for by the fact that this rat had gained so rapidly on 0.1 gm. weekly that it weighed 200 gm. at the onset of the daily feeding period. Certain it is that none of the other rats in this group showed any increased tolerance as a result of their previous weekly dosage, and this rat was not insusceptible, as is shown by his rapid increase in weight after the thyroid was stopped.

Recovery after thyroid feeding is stopped is so rapid that it is worthy of special mention. This was especially noticeable in this experiment, because the rats were about half grown. While being fed thyroid the rats continued their growth but were quite devoid

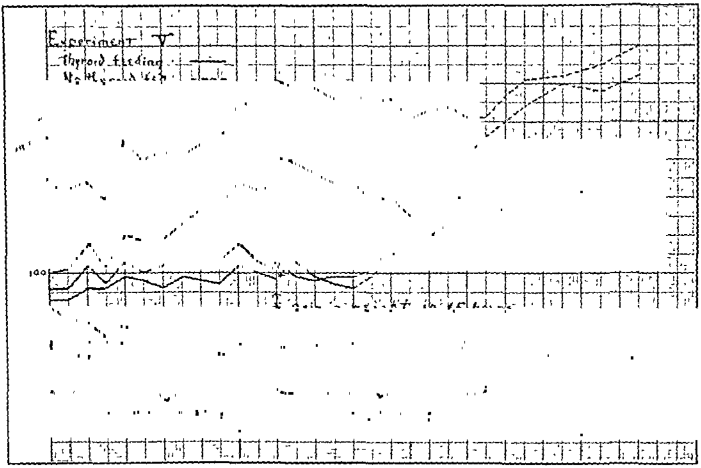

Chart V.

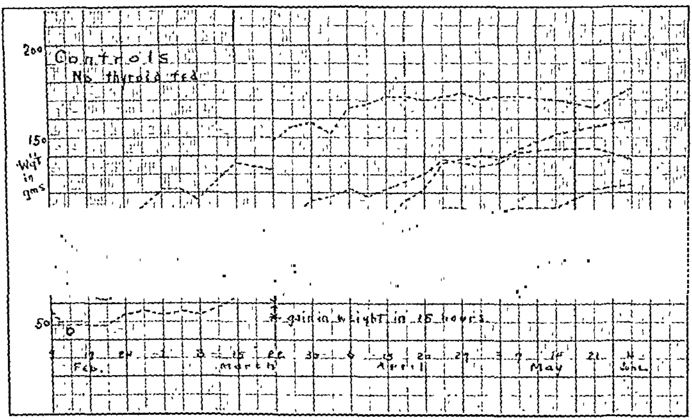

Chart VI.

of fat. This fact, in some of the rats, made the loss of weight appear trifling. For the same reason the gain in weight afterwards was even more dramatic.

There were 27 instances of poisoning in 21 rats with 19 recoveries and 8 deaths. Of those that died, all but one died within thirty hours of the last feeding. One rat lived for a week after the last dose, during which time it had a bloody diarrhea dating from before the last dose of thyroid. (Three rats died in the *second* week of feeding, and these deaths were not considered as due to thyroid.) (See Table I.)

TABLE I

GAIN IN WEIGHT AFTER CESSATION OF THYROID FEEDING

GAIN IN GRAMS	GAIN IN FIRST 3 DAYS	GAIN IN SECOND 3 DAYS	GAIN IN SECOND WEEK	TOTAL GAIN IN 2 WEEKS
10 or less	6 rats	10 rats	8 rats	
10–20	10	9	10	
20–30	3		1	9 rats
30–40				8
40–50				2

ON THE MECHANISM OF THYROID POISONING IN WHITE RATS

Kendall,[2] in 1917, working with goats found that, while 150 mg. of thyroxin by injection could be tolerated at a single dose, 11 doses of 0.1 mg. daily killed, a total dosage of only 110 mg.[3] The same phenomenon has been noted in the present experiment. One-tenth gram of thyroid fed daily (a total of 0.7 gm. weekly) is a positively toxic dose of thyroid in all the nine rats so fed, while 1.0 gm. weekly was tolerated easily and the rats gained in weight. Five-tenths gram twice a week in five rats did not seem toxic, while 0.3 gm. every other day produced poisoning in all five rats and the death of one. Taking a typical rat on the daily feeding of 0.1 gm., it is seen that this rat in twenty-seven days took 2.7 gm. of thyroid and died, while five rats, for the same period of twenty-seven days, took 4.0 gm. of thyroid in four doses at weekly intervals, thriving and gaining in weight during this period.

The toxicity of thyroid for the white rat seems to depend on two factors:

1. The dosage must be adequate. In some of the present experiments 0.01 gm. or approximately $\frac{1}{10,000}$ of body weight, four rats were able to gain some weight, even when fed thyroid for a period over twice as long as that needed to kill when 0.1 gm. daily was used. This was followed by 0.02 and 0.04 gm. daily for a period as long as the fatal period in the other rats without any fatalities. When the dose was increased to 0.07 gm. daily there was distinct poisoning, with one death.

2. The dosage must be of a certain frequency. What this frequency is must be determined by further experiments. Four rats were distinctly poisoned when fed 0.3 gm. every other day, whereas we know that once a week is not often enough.

This is not cumulative action. Cumulative action depends on a faster rate of absorption than the rate of elimination. In all instances of cumulative action it is possible to poison immediately with a large enough single dose. In thyroid intoxication the elimination is rapid, as shown by the very rapid recovery after withdrawal. It may be that the rapid elimination saves the rat, when fed the single large doses, and that the thyroid is eliminated before the tissues have a chance to absorb and be poisoned by it. This does not explain why the tissues are slow in taking up the thyroid when presented in the larger doses.

A somewhat similar action is seen when iodides are administered over a long period in human beings. Iodide can be taken over a period of two or three weeks, and then suddenly acne will develop and this, in spite of the fact that iodide is one of the easiest drugs to eliminate.

The question as to whether this is not a hypersensitive phenomenon might very properly be asked. Anaphylaxis in the dog and rabbit is produced after the repeated injection of small nontoxic doses. In the rat there is, of course, no rapid and dramatic shock as is seen in anaphylaxis, and it is not intended to suggest that we have anaphylaxis here. If this is a form of hypersensitiveness, it is a form which does not appear in any of the classifications of this interesting condition. It also resembles roughly the human form (called by Coca, atopy) as manifested by asthma and hay fever. It is not at all uncommon to find adults of twenty-five and thirty years of age becoming suddenly sensitive to the feathers on which they have slept since childhood. The reason for this development of hypersensitiveness after such prolonged exposure is not at all understood, the only information we have being the hereditary factor demonstrated by Cooke and Vander Veer.[4]

OBSERVATIONS

1. No tolerance to thyroid extract in the white rat has been shown in the present experiments, nor do these experiments entertain much hope that such a tolerance is possible

2 A dose of thyroid, easily tolerated when given in weekly doses, will kill when given in seven doses at daily intervals. Possible explanations of this are: (1) that the thyroid is not absorbed but passes through the bowel unaltered. This does not seem compatible with the digestive powers of the rat. One day the rats were weighed while quite hungry and were then given all the food they would eat and weighed again the next day. The results are shown in the charts as two weights on the same day (indicated by asterisk). It will be seen that there is a gain in weight of from 5 to 15 gm. Since thyroid was usually fed on an empty stomach it does not seem reasonable to suppose that 1 gm of so easily digestible a substance as thyroid would fail to be digested and absorbed. Furthermore, Kendall, working with thyroidin, and Hunt, with thyroglobulin, noticed similar results, and their dosage was administered subcutaneously or intravenously. (2) That the tissues are unable to take up enough thyroid after a single dose before its excretion to produce a toxic effect. (3) That daily dosage of thyroid produces in the tissue a hypersensitiveness to thyroid of a type not at present found in any classification of hypersensitiveness.

REFERENCES

[1]Am. Jour. Phys., 1912, xxx, 129.
[2]Kendall: Mayo Clinic, 1917, ix, 330.
[3]Compare Hunt, Arch. Int. Med., 1925, xi, 682, who notices a different action of thyroid in single large doses and small divided doses in mice.
[4]Jour. Immunol., 1916, i, 201.

THE RENAL FUNCTION INDEX*

A Simple Method for Determining the Functional Activity of the Kidneys

By Adolph Bernhard, Harry G. Jacobi, Friedrich J. Jensen, New York City, N. Y.

VARIOUS methods have been advanced for determining the functional activity of the kidney. Most of them have involved the use of complex formulae, and while at first accepted with great enthusiasm, they have either been discarded or replaced by other formulae of equal complexity.

In 1909 Ambard[1] and his collaborators advanced their ideas regarding the rate of urea excretion, with reference to the concentration of urea in the blood and urine, and proposed the rather complex formula frequently referred to as Ambard's coefficient. In 1915 McLean[2] modified this formula by introducing certain additional factors. In 1921 Austin, Stillman, and Van Slyke[3] published a formula which differed considerably from that of Ambard and McLean, while later, Addis and Drury[4] suggested an index which expressed as a ratio the urea in one hour's urine and the urea in 100 c.c. of blood. This later method, while simple, is not sufficiently accurate because of variations which occur in the hourly urinary excretion.[5]

In 1924 Stander, Duncan, and Moses[6] reported on the excretion rate of urea in the toxemias of pregnancy, using the indices of Addis and Drury[4] and of Stillman, Austin, and Van Slyke.[3] These results were compared with a third formula proposed by them, viz.: $X = \frac{B. \times T.N.}{D}$ *B* represents the urea nitrogen of the blood expressed in milligrams per 100 c.c., *T.N.* the total nitrogen in the urine expressed in grams per twenty-four hours, and *D* the urea excretion expressed in grams per twenty-four hours. The formula of Stander, Duncan, and Moses[6] consists of a ratio, expressing the relation between the blood urea nitrogen and the urine urea nitrogen (expressed as a percentage of the total nitrogen of the urine).

Stander, Duncan, and Moses[6] were unable to obtain uniform results for the urea excretion rate by using the formula of Addis and Drury, but did obtain fairly constant results with that of Stillman, Austin, and Van Slyke. Their new index, however, afforded in general even more constant values than the formula of Stillman, Austin, and Van Slyke.

We have used the index of Stander, Duncan, and Moses in a study of sixty cases of normal and abnormal kidney function in an attempt to establish the normal index and its variations in pathologic conditions.

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PROCEDURE

The urine is collected for twenty-four hours, at the conclusion of which the blood specimen for urea nitrogen (Van Slyke-Cullen) is obtained while the patient is in the fasting condition. The total nitrogen (Kjeldahl), urea nitrogen, and ammonia nitrogen of the urine is determined. During the time of the test the patient is not subjected to any restrictions.

CALCULATION OF THE NORMAL INDEX

A normal adult, of medium size, on a mixed diet, will excrete 12 to 18 grams of nitrogen during twenty-four hours. The urea excretion depends entirely upon the absolute amount of total nitrogen excreted and the mean average daily excretion in normal adults is 80 to 90 per cent of total nitrogen. The blood urea nitrogen in normal individuals varies between 12 to 15 mg. per 100 c.c. of blood. From these assumptions we can calculate the normal index as follows:

If we take the high normal total nitrogen elimination as 18 gm. in twenty-four hours, assume that 80 per cent is excreted as urea (14.4 gm.) and use the high normal blood urea nitrogen of 15 mg. per 100 c.c. in the formula, the index would be 18. Using 12 gm. total nitrogen excretion in twenty-four hours as the low normal, 80 per cent of which (9.6 gm.) is urea and the low normal blood figure of 12 mg. of urea nitrogen, the index would be 15.

It can can be readily seen from the factors entering into the calculation of the index, that several combinations exist in which changes of the index may occur. Only four of these are of clinical interest.

$$A \begin{cases} \text{Increase of blood urea nitrogen} \\ \text{Normal total nitrogen excretion} \\ \text{Normal urea nitrogen excretion} \end{cases}$$

$$B \begin{cases} \text{Normal blood urea nitrogen} \\ \text{Normal total nitrogen excretion} \\ \text{Low urea nitrogen excretion} \end{cases}$$

$$C \begin{cases} \text{Normal blood urea nitrogen} \\ \text{Low total nitrogen excretion} \\ \text{Low urea nitrogen excretion} \end{cases}$$

$$D \begin{cases} \text{Increased blood urea nitrogen} \\ \text{Decreased total nitrogen} \\ \text{Decreased urea excretion} \end{cases}$$

We are chiefly concerned with types *A*, *C*, and *D*, while *B* is the type not encountered in nephritis but in cases of poor liver function or improper urea formation.

CLASSIFICATION OF CASES

Arbitrarily the cases have been classed in three groups according to the index obtained. The first group comprises those with an index up to 18 (the normal group), the second from 18 to 40, and the third over 40. The first group was further subdivided into those cases having a normal index, negative urine and no hypertension (Table I-*A*) and those cases with a normal index, albumin and casts in the urine and hypertension (Table I-*B*).

Group two (Table II) cases show an index between 18 and 40. It is in this group (thirty cases) that the so-called mild nephritics, with normal blood chemical findings, are to be encountered. In several of the patients of this group, follow-up determinations of the renal function index were made at varying intervals after treatment, in order to determine whether clinical improvement was accompanied by a similar improvement in the index. It would be without purpose to discuss all of the thirty cases. Several selected ones will be sufficient to give the general idea.

CASE 6726.—A painter, age thirty-six, complained of pain and swelling of the left knee-joint, which had been preceded by a sore throat, cough and fever. His past history revealed that for several years he had suffered from shortness of breath, particularly after exertion. His physical examination showed carious teeth, enlarged heart with double murmur at the apex, and tenderness of both knee-joints. There was also a generalized glandular enlargement. The diagnosis of acute rheumatic fever with chronic endocarditis was made.

On November 11, 1924, the chemical blood examination showed: urea nitrogen 15.6, creatinine 2.3, uric acid 3.3, sugar 138. The urine at this time was negative. On January 6, 1925, another blood examination showed that the blood urea nitrogen had increased from 15.6 to 20.8 and the renal function index determined on that day was 33.4. The urine showed the presence of albumin and hyaline casts. The patient was then placed on a restricted protein diet with rest in bed for about ten days. His rheumatic fever had subsided and he was discharged in good condition. On March 16, the patient returned for another determination of the index. He reported that he had been able to carry on his usual work since discharge and had enjoyed good health. No special restrictions in the diet had been enforced. The blood urea nitrogen at this time was 8.9 and his index had decreased to 11.3. The urine examination was negative.

CASE 7244.—A clerk, age forty-six, gave the following history: A few days prior to admission, he caught cold and developed an acute otitis media. Following this he began to complain of chills and had rusty sputum. His past history was that of scarlet fever during childhood, chancre thirty years ago, and an attack of influenza about four years before admission. He had also been subject to frequent attacks of sore throat. The patient presented a typical pneumonic consolidation of the left upper lobe.

On January 5, 1925, two days after admission, a chemical blood examination revealed a urea nitrogen of 35.7, creatinine 1.3, uric acid 3.5 and sugar 162. On January 8, the

TABLE I-A

NORMAL INDEX, NEGATIVE URINARY FINDINGS

HIST. NO.	DATE	URINE		BLOOD		URINE		DIAGNOSIS	DIET	INDEX
		U. N.	T. N.	UREA N.	PRESSURE	ALB.	CASTS			
6350	11/25	6.74	8.2	14.2	124/76	0	0	Syphilis	Soft	17.2
6894	12/4	7.6	10.5	11.5	104/64	0	0	Duod. Ulc.	Ulcer Diet	15.8
3663	12/6	4.81	4.97	16.6	112/70	0	0	Lung Abscess	Soft	17.1
6954	12/9	5.9	6.2	7.2	120/90	0	0	Neurosis	Norm.	7.4
6698	12/15	2.95	4.47	8.9	110/80	0	0	Arthritis	Soft	13.4
6762	12/20	4.9	7.03	10.0	90/60	0	0	Duodenal Ulcer	Ulcer Diet	14.3
	1/13	5.7	7.0	11.4	"	0	0	" "	"	14.0
7223	1/5	7.62	8.2	10.5	100/64	0	0	Hodgkin's	Full	11.2
7155	1/12	6.5	9.0	12.5	104/80	0	0	Convalesc. Pneumonia	Semi-solid	17.2
1571	1/15	6.4	10.0	11.4	138/80	0	0	Chr. Valv. Disease	Soft	17.8
Mrs. J.	1/31	9.4	11.8	9.2	120/80	0	0	Pregnancy	Norm.	11.5
8115	4/6	5.4	7.2	12.5	150/90	0	0	Ac. Rheum. Fever	Soft	16.6
6730	4/25	5.0	5.9	14.8	102/65	0	0	Nephritis	Milk	17.4

TABLE 1-B

NORMAL INDEX, POSITIVE URINARY FINDINGS

HIST. NO.	DATE	URINE U. N.	URINE T. N.	BLOOD UREA N.	BLOOD PRESSURE	URINE ALB.	URINE CASTS	DIAGNOSIS	DIET	INDEX
7105	1/15	2.7	4.0	10.9	170/64	+++	Hyal. Gran.	Chronic Nephritis	Low Prot.	16.1
7537	2/6	8.1	8.5	9.6	110/80	+	Hyal. Gran.	Chr. Valv. Disease	Karell	10.2
7580	2/11	11.3	13.1	12.5	180/100	+	Hyal.	Chr. Nephritis	"	14.5
7633	2/13	5.5	7.4	8.0	225/120	++	Hyal.	Cardiorenal Dis.	Soft	10.8
7599	2/28	10.3	12.5	13.0	160/90	+	Gran. Hyal.	Chronic Nephritis	"	15.7
7751	3/5	4.2	5.9	12.5	150/100	+++	Gran. Hyal.	Cardiorenal Dis.	"	17.5
7808	3/6	6.5	8.4	13.0	115/75	+	0	Hyperthyroid	"	17.5
5925	3/27	4.8	5.5	9.2	180/110	+	Hyal.	Cardiac Insuffic.	Milk	10.5
8018	3/31	9.5	12.9	14.7	105/65	+	0	Tbc. Pneumonia	Soft	17.8
7986	3/31	7.4	8.6	12.5	125/55	+	Hyal.	Hyperthyroidism	"	14.5
7944	4/9	3.4	4.2	13.8	110/60	+	Hyal.	Acute Rheumatic Fev.	Fluid	17.0
Dr. D	11/8	5.22	7.31	12.6	120/80	+	Hyal.	Normal	Norm.	17.7

temperature returned to normal by crisis and two days later the renal function index was determined. This was found to be high (28.2), although the blood urea nitrogen at this time had decreased to 20.9 The urine showed the presence of albumin but no casts. On April 3, 1925, three months later, the renal function index was normal (13.6) and the blood urea nitrogen was also normal (11.5).

CASE 7219.—A female, age thirty-nine, was admitted, complaining of difficulty in urination and pain in the lumbar region following a "heavy cold," also a sore throat and cough. During childhood she had had scarlet fever and frequent attacks of sore throat. In 1918 she had influenza, and a year before admission she had her tonsils removed. For the past few years she suffered considerably from severe headaches and impairment of vision. The diagnosis of acute nephritis with pharyngitis and bronchitis was made. The patient had badly infected teeth, inflamed throat, tenderness over the left kidney region and an albuminuric neuroretinitis. The blood pressure was systolic 130, diastolic 80, and the urine showed the presence of albumin and casts.

Chemical blood examination on January 2, 1925, showed urea nitrogen 18.5, creatinine 1.0, uric acid 3.3, sugar 90. These findings do not indicate retention of the nitrogenous constituents of the blood. On January 12, the renal function index was found to be definitely increased (22.3), while the blood urea nitrogen was still normal (17.9). After several weeks the patient was discharged greatly improved. On March 12, the patient reported that she did not have any of the symptoms previously mentioned and the renal function index had returned to normal (12.5). The blood urea nitrogen was 10 mg. per 100 c.c.

CASE 7295.—A watchman, age fifty-nine, was admitted, complaining of pain along the course of the right sciatic nerve, of two weeks' duration. Previous history was that of a gonococcus infection eighteen years ago. The tonsils had been removed thirty years ago and all the teeth had been extracted four years ago.

On January 12, the blood urea nitrogen was 15.6 mg. and the renal function index was 23.7. The urine was repeatedly negative and the blood pressure normal. The patient was kept in bed and given a soft low protein diet. February 18, a second examination showed the blood urea nitrogen to be 12.5 mg. and the index 20.4. The patient was discharged greatly improved. On March 31 the blood urea nitrogen was 14.7 and the functional index still high (20.4), showing that according to the functional index, the patient still had some impairment of renal function, although the blood figure still remained normal.

CASE 7392.—A man, age twenty-nine, complained of pain in the chest, cough, fever, and headache of ten days' duration. He had also had several attacks of vomiting during this time. For the previous three years he had nocturia, and for the past four months,

TABLE II

CASES SHOWING INDEX BETWEEN 18 AND 40

HIST. NO.	DATE	URINE		BLOOD		URINE		DIAGNOSIS	DIET	INDEX
		U. N.	T. N.	UREA N.	PRESSURE	ALB.	CASTS			
6986	12/12	5.5	7.6	14.2	110/66	0	0	Acute Rheumatic Fever	Soft	19.5
6726	1/6	6.1	9.8	20.8	104/66	+	Hyal.	Ac. Rheum. Fever	Soft	33.4
[illegible]	3/16	9.0	11.5	8.9	110/75	0	0		Norm.	11.3
Dr. J.	1/7	10.6	14.3	16.6	130/85	0	0	Normal	Norm.	22.3
7244	1/10	5.1	6.9	20.9	130/90	+	0	Lobar Pneumonia	Soft	28.2
	4/3	9.6	11.5	11.5	120/80	+	Hyal.		Norm.	13.6
7219	1/12	7.2	9.5	17.9	130/80	+	Hyal.	Acute Nephritis	Soft	22.3
	3/12	5.1	6.9	10.0	120/75	0	0		Norm.	13.5
7295	1/12	7.5	11.4	15.6	124/64	0	0	Neuritis	Soft	23.7
	2/18	6.8	11.1	12.5	120/70	0	0		"	20.4
	3/31	8.2	10.1	14.7		0	0		Norm.	18.1
9014	1/15	8.7	11.7	16.7	130/60	0	0	Broncho-pneumonia	Soft	22.4
7284	1/15	5.3	7.5	11.7	110/70	0	0	Chr. Myocarditis	Salt Free	23.6
7392	1/25	9.4	10.6	35.7	162/104	+	Hyal. Gran.	Grippe Ac. Neph.	Low Prot.	39.4
7268	1/25	5.03	9.7	10.0	104/90	+	Hyal.	Pylorospasm	Soft	19.2
7413	1/27	3.8	4.2	36.0	—	+	Hyal. Gran.	Acute Nephritis	Low Prot.	39.5
	2/12	4.47	5.3	31.2	—	+	Hyal. Gran.	"	"	37.0
	2/19	3.9	4.0	12.5	110/80	+	"	"	"	12.8
3604	2/4	7.23	11.1	12.5	150/75	+	Hyal.	Cirrhosis Chr. Myoc.	Soft	19.2
7736	2/26	6.0	9.4	18.0	155/100	+	Hyal.	Cirrhosis Chr. Myoc.	Soft	28.2
7524	2/28	8.6	14.3	14.7	116/78	+++	Hyal.	Chr. Myoc.	Soft	24.4
7745	3/4	14.1	15.6	16.6	120/75	+	Hyal.	Peptic Ulcer.	Spec. Ulcer	18.3
7729	3/6	6.3	8.0	15.6	135/85	0	0	Pleurisy	Soft	19.8
7516	3/9	7.5	9.6	17.8	145/95	++	Hyal.	Hematuria Rt. Kidney	Soft	22.6
7887	3/11	5.8	7.5	14.0	140/75	++	Hyal.	Acute Nephritis	Mod. Karell	18.1
7471	3/14	7.0	9.6	31.2	190/85	+	Hyal.	Cardiac Nephritic	Karell	22.7
7933	3/17	5.6	8.2	17.8	180/100	++	Hyal.	Cardiac Decomp.	Karell	26.0
7930	3/18	4.8	8.7	10.4	115/55	+++	Pus Cells	Calculus (Renal.)	Soft	18.8
7989	3/23	5.1	10.0	10.0	150/15	++	Hyal.	Chronic Endocard.	Soft	19.6
7174	3/23	4.9	7.6	17.0	170/85	+++	Hyal.	Chronic Cardiac	Soft	26.3
8019	3/27	5.3	9.6	11.4	90/40	0	0	Diabetes	Spec. Diabetic	20.6
"	4/6	4.6	6.0	9.2	"	0	0	"	"	12.0
8031	3/30	6.6	9.4	25.0	180/65	+	Hyal.	Broncho-pneumonia	Karell	35.6
7804	3/30	8.2	12.0	17.0	165/90	++	Hyal.	Chronic Nephritis	Soft	24.8
7991	4/7	5.9	9.2	12.5	115/85	++	Hyal.	Emphysema Chr. Myoc.	Soft	20.1
8203	4/14	4.7	7.6	17.0	133/75	+	Hyal.	Chronic Myocard.	Karell	27.5
7876	4/25	4.5	6.4	13.2	120/75	+	Hyal. & Gran.	Acute Nephritis	Liquid	18.7
8305	4/24	4.8	6.2	16.6	130/75	++	0	Impacted Feces	Soft	24.2

TABLE III

INDEX ABOVE 40

HIST. NO.	DATE	URINE		BLOOD		URINE		DIAGNOSIS	DIET	INDEX
		U. N.	T. N.	UREA N.	PRESSURE	ALB.	CASTS			
6601	11/3	1.49	2.41	125	156/88	+++	Gran. Hyal.	Chronic Nephritis, Uremia	Low Prot.	202.1
6955	12/8	5.4	10.27	22.7	130/90	+	0	Arterio-sclerosis	Soft	43.2
7245	1/10	5.96	9.0	41.6	180/40	+	Gran. Hyal.	Chr. Neph. Chr. Myoc.	Karell	62.8
7730	4/4	6.7	13.1	19.0		+	Hyal.	Rheumatic Fever	Soft	50.4
7392	4/9	4.9	6.9	62 5	205/125	+++	Gran. R. B. C.	Grippe Ac. Neph.	Karell	88.0
7929	4/16	5.7	15.9	22.7	165/100	+++	Hyal.	Acute Nephritis	Karell	63.3

pain in both kidney regions. Physical examination disclosed an acutely inflamed throat and fever. The diagnosis of influenza was made. Eye-ground examination showed a retinitis with tortuosity of the blood vessels, as seen in cases of hypertension. The blood pressure was systolic 205, diastolic 125. The urine showed the presence of albumin and hyaline casts.

On January 17, 1925, a chemical blood examination gave the following values: urea nitrogen 50 mg.; creatinine 1.4, uric acid 6.0, sugar 141, and carbon dioxide combining power of the blood plasma 48.1 volume per cent, per 100 c.c. January 21, the chemical blood examination was repeated with these results: urea nitrogen 45.6, creatinine 4.5, uric acid 4.9, sugar 119. The increase in the creatinine content pointed to kidney impairment. On January 25, the renal function index was found to be 39.4. The patient was put on a low protein diet and kept in bed. April 9, (see Table III) the index was again determined and found to have increased to 88.0. Clinically, the patient was much worse.

CASE 7413.—This case was an acute hemorrhagic nephritis in which the renal function index was determined at varying intervals. On January 27, the blood urea nitrogen was 36.0 and the index 39.5. The patient was placed on a low protein diet and on February 12, the index was 37.0. One week later the index was again determined and it was found to be 12.8. Clinically, the patient showed marked improvement, while his urine still contained albumin and casts.

CASE 8019.—A man, age twenty-eight years, was admitted on March 23, 1925, with diabetic coma. Patient was first told of his diabetic condition three years prior to present admission. Two years ago he had swollen legs which lasted several weeks. On March 27, four days after admission the renal function index was 20.6 with a blood urea nitrogen of 11.4. On March 30, slight puffiness of the face was noted and on the following day this had extended, causing marked swelling of the face and extremities. Under proper diet and rest in bed the edema cleared up. Urine examinations were at all times negative for albumin and casts. On April 6, a second renal function test was done and this time the index was 12.0.

Group 3 (Table III) cases showing an index above 40. There were six cases in this group, of which one was a repetition of a case included in Group 2. The urinary and blood findings and clinical course in all these cases are so definitely indicative of renal impairment, that it would hardly be worth while to enter into any further detailed discussion of them.

DISCUSSION

Chemical examination of the blood is of great value in determining the retention of waste nitrogenous constituents. Such examination, however, gives no indication of the excretory capacity of the kidney. It has been the experience of all who have observed any considerable number of cases to

meet with instances in which the blood chemistry has been normal up to within a few hours previous to death, while the clinical evidence supported by postmortem examination, warranted a diagnosis of severe nephritis. Again the blood chemical examination may give results which are considered as high normal or only slightly increased above normal, and no definite statement can be made as to the presence or absence of a nephritis, or as to its severity.

As previously stated, the normal eliminating power of the kidney as regards the urea nitrogen is 80 to 90 per cent of the total nitrogen. This percentage elimination is diminished in nephritis, usually before definite changes in the blood nitrogenous constituents are obtained. An example of this decreased eliminating power of the kidney is encountered in mechanical obstruction, as in ligation of the ureters, and in acute conditions such as mercury, uranium or tartrate nephritis, where the decreased elimination is the first symptom, after which retention of the waste nitrogenous constituents of the blood results.

It would seem that any procedure involving a study of both the elimination of nitrogenous constituents and their concentration in the blood, would give a more accurate estimate of the degree of renal function. The present index offers such a combination of methods. The procedure has the advantage of being simple in technic, of imposing no hardship on the patient, and of lending itself to routine procedure. Experience indicates that the renal function index may be helpful in the diagnosis of doubtful cases of nephritis.

SUMMARY

1. The index of Stander, Duncan and Moses has been studied in 60 cases.

2. The normal index has been found not to exceed 18.

3. An index between 18 and 40 is indicative of impairment of renal function, although the blood figures may be within normal limits.

4. Changes in the index in nephritis may be of aid in prognosis.

5. In severe cases of nephritis, indices higher than 40 have been obtained, one case reaching 202.

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A STUDY OF THE ANTIGEN USED IN THE WASSERMANN TEST FOR SYPHILIS*

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I. THE RÔLE OF THE ANTIGEN IN THE FIXATION OF COMPLEMENT AND IN THE FORMATION OF A PRECIPITATE

DESPITE the immense amount of work that has been done in the endeavor to find out how the antigen brings about complement fixation in the presence of syphilitic serum, the exact nature of the Wassermann reaction is still a mystery. One thing, however, is certain, that the reaction is not due to a specific antigen-antibody union. This view was strengthened by the findings of Schereschewsky[1] and Noguchi[2] that a specific antigen prepared from cultures of Spirochete pallida cannot be used as successfully in the diagnosis of syphilis as antigens prepared from tissues of normal organs.

A number of theories have been advanced to account for the Wassermann reaction.

Noguchi[3] demonstrated that the active antigenic principles in normal tissues were lipoids, and he concluded that the reaction depends upon lipotropic substances present in the patient's blood serum. He also claims[4] that there exists a certain relationship between the iodine number of an antigen and its binding power. This seems to indicate that a chemical union may take place at the free bonds of the unsaturated carbon atoms in the lipoid molecule with the substances present in the syphilitic serum, and a deviation of complement is brought about when this union has taken place.

Manwaring[5] advanced the opinion that there is a proteolytic ferment present in the guinea-pig's blood serum, which has the power to destroy complement. The fixation of complement is due to substances present both in the antigenic extracts and in the patient's serum, which bring about favorable conditions for the activation of the ferment.

Levaditi and Yamanouchi[6] believe that the reaction depends upon the union of two colloidal factors, one consisting of the lipoidal substances in the antigen, and the other present in the patient's blood. Both substances, they believe, are alcohol-soluble and nonprotein in nature.

Schmidt[7] considers the reaction colloidal in nature and believes that it depends upon the union of the colloids in the antigen with the globulins in the patient's serum. These globulins are increased in amount and changed in nature in syphilitic patients. No such union occurs in normal serum, it being prevented by the albumins which act as protective colloids.

Citron,[8] and Weil and Braun[9] forwarded somewhat similar theories, the so-called "auto-antibody" theories, both resting upon the belief that in the blood of syphilitic persons antibodies to the lipoids in the antigenic extracts

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exist; these antibodies being produced by the absorption of albumin-lipoid substances, elaborated by the growth of Treponema pallidum, and the subsequent production of antibodies against these substances.

Porges and Myer[10] were the first to observe that a precipitation occurred when a lecithin emulsion was added to syphilitic serum.

Many workers, therefore, are of the opinion that the Wassermann reaction is due primarily to the precipitation of globulins by the lipoidal colloids of the antigen, and that the resulting precipitate is capable of absorbing complement; thus the removal of complement is dependent upon the precipitate formation.

None of the theories, however, rest upon sufficient experimental evidence to be accepted as the true explanation of the process of complement fixation in syphilis.

Whether the deviation of complement actually depends upon the formation of a precipitate by the combination of the antigen with syphilitic serum has not really been demonstrated. Hull and Faught[11] attempted to separate the precipitate formed in the "Sachs and Georgi" test[12], and to determine whether it is entirely responsible for the Wassermann reaction. They centrifuged the precipitate at high speed for an hour and performed a complement-fixation test on both the precipitate and supernatant fluid. They obtained a positive Wassermann test with both the precipitate and the supernatant fluid, thus obtaining inconclusive results.

Dean[13] found that the proportion of antigen and antibody favorable for rapid and complete precipitation does not favor the most complete complement fixation.

In a previous paper,[14] I have shown that the antigenic substances in beef heart are thermostabile and can withstand boiling or even heating in the autoclave under 15 pounds pressure for an hour without impairing their antigenic properties. Accordingly, I have described a method for preparing an antigen by using high temperatures. The antigen thus prepared gave good results when used in the Wassermann test. When I attempted to use this antigen in the precipitin test according to the method of Sachs and Georgi, however, I obtained negative results. The explanation for this was that the precipitating property of the antigen was destroyed by the heat employed in the preparation of the antigen. To prove that such was the case, two identical antigens were prepared, except that one was made at room temperature while the other was heated. The results were, as expected, that the antigen prepared at low temperature gave heavy precipitates with positive syphilitic sera, while the antigen prepared at high temperature gave no precipitates whatever.

Thus it seems that complement fixation does not depend upon the formation of a precipitate; the two phenomena do not run parallel courses, but they probably represent different phases of the same phenomenon. Yet, one cannot accept this as truth with any degree of certainty, for failure to notice a precipitate macroscopically does not prove the absence of an invisible precipitate: we know that albumin particles may aggregate into larger particles without causing precipitation, provided the excess of one of the precipitate-forming colloids acts as a protective colloid.[15] Thus, further evidence is necessary to settle this question.

II. THE LIPOIDAL NATURE OF THE ANTIGEN

A question of great importance is whether the antigen is a pure lipoid or a lipoid-protein combination. According to Landsteiner,[16] mere solubility of a substance in organic solvents cannot be taken as proof of its lipoidal character. It is very probable that nonlipoidal substances may go into solution in organic solvents when lecithin is present. Michaelis and Rona[17] have shown that alcoholic lecithin solutions protect albumoses from the precipitating action of alcohol. Müller[18] found that the antigen of typhoid bacilli is soluble in chloroform, in the presence of old preparations of lecithin. Thus, there is a possibility that the antigenic property of the alcoholic extracts used for complement fixation in the Wassermann test is due to a lipoid-protein combination which permits the presence of protein, mechanically or chemically united to the lipoid in the alcoholic solution.

Since mere extracting with fat solvents does not separate all the protein from the lipoids, other means have to be applied for removing all traces of protein from lipoids. To accomplish this I have digested beef heart muscle with trypsin previous to extraction with alcohol. Trypsin was chosen in preference to pepsin because its digestive power is more complete, reducing protein to amino acids, and also because it works in a neutral medium.

Procedure.—Trypsin was prepared according to the method of Stutzer and Merres,[19] which is as follows:

A calf pancreas was freed from fat, ground through a meat grinder, and crushed with sand in a mortar. This was left in the mortar for twenty-four hours (during this period the trypsinogen is converted into trypsin). Water was then made alkaline with lime, and three parts added to one part of pancreas (without the sand) and one part glycerine. The mixture was allowed to stand for four days. The mass was then squeezed through flannel, filtered through filter paper and evaporated to dryness in a vacuum.

The reaction of the trypsin was neutral to litmus. The lime was added to saponify fat and to destroy lipase. (Too much lime weakens the tryptic activity.)

Fifty grams of beef heart were ground up and divided in two equal parts. To one portion was added 100 c.c. of water and 0.25 grams of trypsin. This was digested for six hours on the water-bath at 38° C. The tissue dissolved and gave no biuret test. It was then evaporated to dryness on the water-bath. The ether-soluble substances were now removed by extracting with ether in a Soxhlet apparatus, and the residue was extracted for two hours with 25 c.c. of hot alcohol.

The other portion of the beef heart was dried on the water-bath, extracted with ether, and the residue extracted for two hours with 25 c.c. of hot alcohol; thus the two antigens were prepared identically with the exception of the tryptic digestion, the last one serving as a control.

Titration of Antigens.—The antigens were titrated with an antisheep hemolytic system, one-fourth Wassermann quantities being used. The complement was titrated immediately before use. Two units of complement and two of amboceptor were used. Complement fixation was done in the ice box for four

hours; sensitized red cells were then added and the second incubation was done in the water-bath at 37° C. for twenty minutes and the results read.

The antigens were made up in two dilutions: 1 to 10, and 1 to 40, by slowly adding saline from a pipette to a measured amount of antigen into a flask, shaking it gently all the while.

A series of 13 tubes were set up, the first four tubes receiving increasing amounts of the 1 to 40 diluted antigen, and the remaining tubes receiving increasing amounts of the 1 to 10 diluted antigen as shown in Table I.

TABLE I

TITRATION OF ANTIGENS

	BINDING POWER						ANTICOMP. PROPERTY					HEMOLYTIC POWER	
TUBE	1	2	3	4	5	6	7	8	9	10	11	12	13
1/40 Ant. c.c.	.01	.02	.05	0.1									
1/10 Ant. c.c.					.05	0.1	0.2	0.5	.75	1.0	1.2	0.5	1.0
Final dilution	1/4000	1/2000	1/800	1/400	1/200	1/100	1/50	1/20	1/13	1/10	1/8	1/20	1/10

The first six tubes contain antigen in dilutions ranging from 1-4000 to 1-100; they all contain 0.05 c.c. positive serum each, thus serving as a test for the antigenic power of the antigens.

Tubes 7 to 11 inclusive contain no serum (except tube 7) and serve to demonstrate the anticomplementary property.

Tube 7 contains 0.1 c.c. of negative serum and serves to test the specificity of the antigens.

The last two tubes contain no serum and no complement, and show the hemolytic power of the antigens.

The following signs were used in writing the results: ++++ = complete inhibition of hemolysis; +++ = slight hemolysis; ++ = half inhibition; + = slight inhibition; 0 = complete hemolysis.

TABLE II

RESULT OF ANTIGEN TITRATIONS

ANT. DILUTION	1-40				1-10								
PROPERTIES	ANTIGENIC						ANTICOMP.					HEMOLYTIC	
TUBES	1	2	3	4	5	6	7	8	9	10	11	12	13
Ant. c.c.	.01	.02	.05	0.1	.05	0.1	0.2	0.5	.75	1.0	1.2	0.5	1.0
Trypsin digested	0	0	++	++++	++++	++++	0	0	0	+	++	++++	++
Control	0	0	+++	++++	++++	++++	0	0	0	++	+++	++++	++++

As shown in Table II the digestion of the protein did not destroy the antigenic properties of the beef heart, thus indicating that the active principle in the antigen used in the Wassermann test for syphilis is not a protein nor a protein-lipoid combination, but a pure lipoid.

SUMMARY

1. Heating of the beef heart tissue or of the alcoholic extracts used as antigens in the Wassermann test for syphilis does not affect their complement-fixation power but does destroy their precipitate-forming property. Whether

this shows that the precipitate formation and complement fixation are two distinct and separate phenomena cannot be told, for failure to see a precipitate macroscopically does not prove the absence of an invisible precipitate.

2. The digestion of beef heart muscle with trypsin in neutral solution does not injure the antigen; hence the antigenic substances are true lipoids and not combinations of lipoids with proteins.

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PROGNOSTIC VALUE OF EOSINOPHILES IN CASES OF EAR INFECTION*

By Viola Brewer, Ph.G., B.S., Spokane, Wash.

Much has been said as to the value of the leucocyte and differential count as an aid to prognosis, but I have seen very little concerning the value of the eosinophiles.

In our general hospital laboratory, we have been called upon to make a leucocyte and differential count daily for several days, and sometimes for weeks, for patients suffering from acute or chronic ear infections. It has been noticed that when the infection is subsiding the eosinophiles appear in the blood stream, together with a lower leucocyte count. The temperature also becomes lower at the same time.

I have made a study of fifty hospitalized cases of this nature for whom two or more leucocyte and differential counts were made. Of these counts, 130 showed eosinophiles and 181 did not show any. The following work has been based upon the 130 differential counts which showed eosinophiles.

As observed from Chart I, the greatest percentage of eosinophiles occurs when the temperature is between 98° and 100°, i.e., near normal. In those cases where a temperature of 104° to 106° was attained, however, the eosino-

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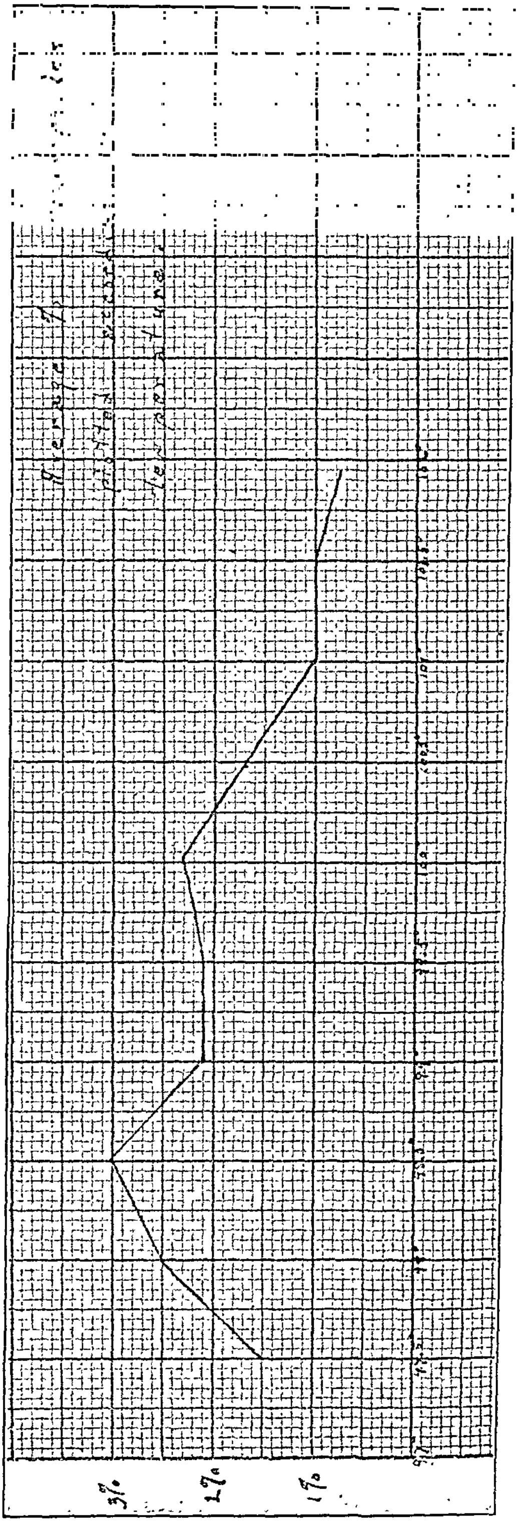
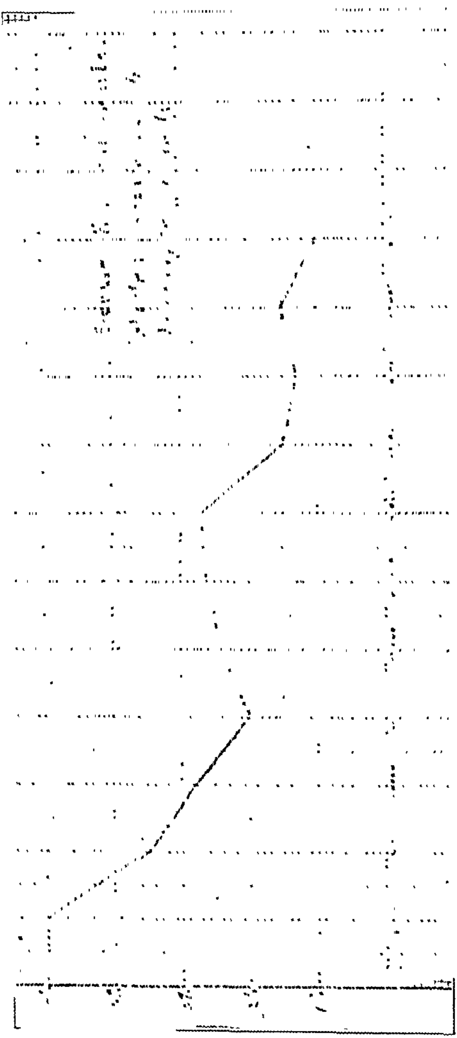


Chart I.



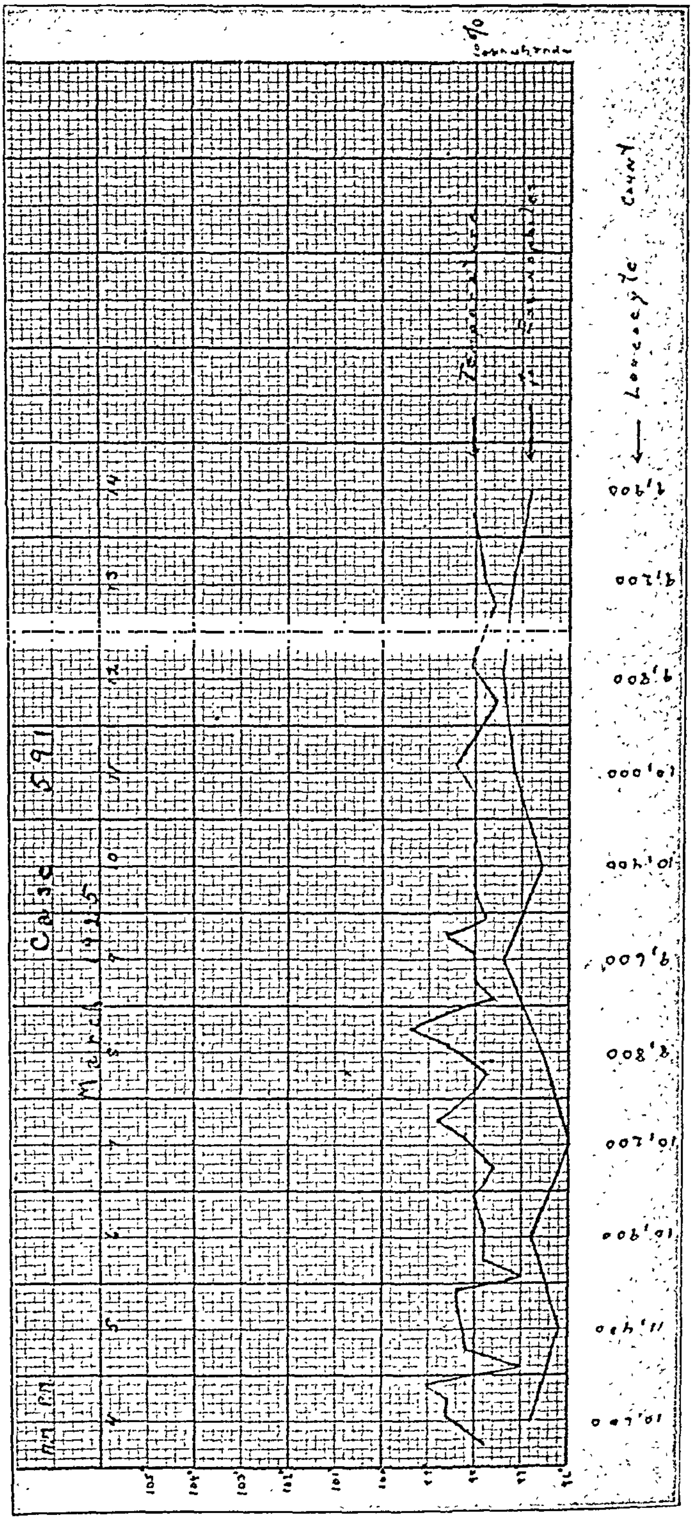


Chart III.

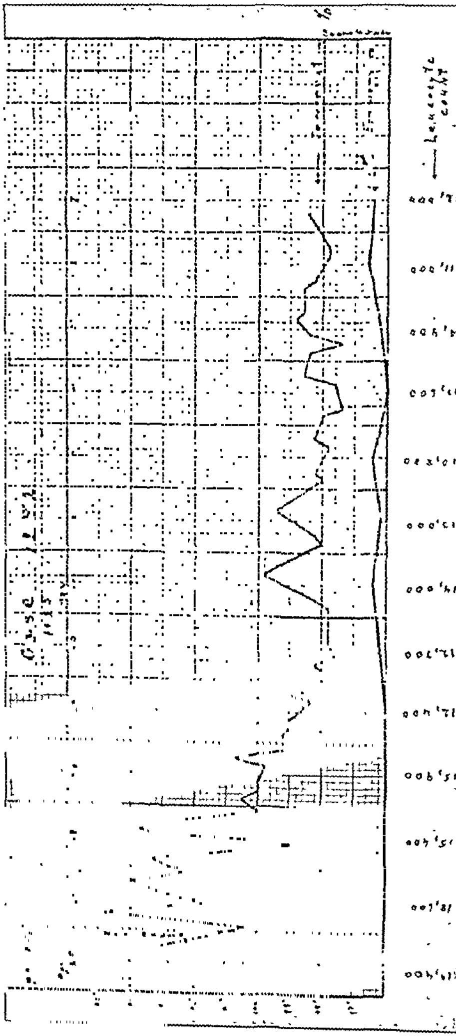


Chart IV.

philes appeared almost simultaneously with a decline in temperature and a subsidence of the clinical symptoms, so, in a few cases, we found 1 per cent or 2 per cent of eosinophiles accompanying a temperature of 103° to 104°. These were cases in which the clinical symptoms also indicated improvement. In no case did eosinophiles occur just previous to a relapse or when the temperature was markedly subnormal.

Chart II illustrates the curve when the eosinophiles are considered with relation to the leucocyte count. We find that the same is, in general, true. The greatest percentage of eosinophiles occurs when the leucocyte count is between 8,000 and 14,000, although, in some cases that had been running a very high count, 1 per cent or 2 per cent occurred as high as 25,000. Here too, the patient showed an improvement in temperature and other clinical symptoms.

The highest per cent of eosinophiles recorded was 10 per cent in a chronic case, eight hours after drainage was established. As long as there was free drainage and the temperature and leucocyte count were near normal, 3 per cent to 6 per cent was quite common.

Of the 50 cases studied, 19 underwent mastoid and 9, myringotomy operations. The occurrence of eosinophiles was the same whether an operation was performed or the patient recovered without an operation. They appeared when the patient was convalescing and disappeared when there was a relapse.

Chart III illustrates the curve of temperature and eosinophiles, with leucocyte count noted, in the typical case of a man with acute purulent otitis media who recovered without submitting to an operation.

Chart IV is that of a boy who underwent a simple mastoidectomy and recovered with no complications.

Both of these cases were selected at random and illustrate the fact that eosinophiles appear in the differential count when the patient's condition is improving.

CONCLUSIONS

1. Eosinophiles occur in the differential count of patients suffering from ear infections: (a) when the temperature is on a downward trend and nearing normal but not subnormal, (b) when the leucocyte count is tending toward normal and the clinical indications are favorable.

2. Eosinophiles are a favorable prognostic sign when taken into consideration with the leucocyte count and the clinical symptoms.

THE ANTISCORBUTIC VITAMINE IN FRESH BEEF*

By Grace Medes, Minneapolis, Minn.

IN VIEW of the repeated statements that fresh meat will prevent scurvy in man,[1] various attempts have been made to test the assertion by experiments with guinea pigs. Chick, Hume and Skelton[2] obtained no protection when they fed guinea pigs with 10 c.c. of fresh raw beef juice daily. Pitz[3] believed that death of guinea pigs was delayed by feeding dried meat, but Dutcher, Pierson and Biester,[1] Chick, Hume and Skelton,[2] and Givens and McCluggage,[4] ascribe the delay of death in his experiments to the fresh milk which was supplied in unlimited amounts. Dutcher, Pierson and Biester, in the same article,[1] show that aqueous extracts of fresh beef do not prevent scurvy. In another instance they fed 5 grams of chopped raw beef daily, with similar result. Givens and McCluggage[4] fed 1.5 grams of meat dehydrated *in vacuo* at a temperature not exceeding 65° for a period of twelve hours, and found that the onset of scurvy was not delayed.

The experiments quoted above seem incomplete on two points. First, in the case of meat extracts or expressed juice, it may be that the vitamine is retained in the meat residue. Second, in those instances in which fresh meat itself was fed, it is possible that the amount consumed daily was too slight to afford protection to an animal requiring as large an amount of the antiscorbutic factor as the guinea pig.

The first series of experiments was begun during the summer of 1920 and was previously published.[5] It was hoped that the guinea pigs could be induced to eat fresh meat in larger amounts than they had in any of the previous experiments. Fresh beef was secured daily from a meat market and was kept on ice until time of feeding. The animals were fed three times daily and at each feeding the meat was freshly scraped or ground in a meat chopper, as it was found that after it had been ground for a few hours the guinea pigs would not touch it. The ground beef was then stirred with a small amount of alfalfa meal and wheat flour, which had been previously mixed in equal parts by weight. The whole was then made into a paste-like ball with water, and rolled in alfalfa meal. Each animal was given daily 2 grams of milk powder stirred with water. Young guinea pigs were selected, ranging in weight from 100 to 250 grams in weight, as the younger animals could be trained more successfully to eat the beef. By observing these precautions, the guinea pigs were induced to consume an average of 15 to 30 grams of fresh meat daily. The alfalfa meal, milk powder and flour were tested on guinea pigs, and at that time were declared to be devoid of vitamine *C*.

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Twelve guinea pigs were used in the experiment. Two served as controls, one of which died of scurvy on the thirty-fifth day and the other of which was killed and autopsied on the twenty-sixth day. Both showed lesions of scurvy. Three refused to eat the meat and died, probably of starvation, after about a week. Five guinea pigs on the meat diet died of scurvy on the twenty-third, twenty-fourth, twenty-fifth, and twenty-sixth days, respectively. Two received orange juice with their food after the twenty-fifth day when they seemed to be dying of scurvy. Both recovered completely and remained healthy on the meat diet plus orange juice, showing that the meat diet is not, in itself injurious to guinea pigs. As may be seen, no protection was afforded.

The following winter the experiment was repeated, modified in several respects. In the experiment described above, the control animals received alfalfa meal in larger amounts than those fed on meat. Since they outlived the latter, the question arose as to the possibility that the alfalfa meal contained traces of the antiscorbutic vitamine. In the following experiment, the alfalfa meal was weighed daily and the three animals received equal amounts. The milk powder was heated in an oven at 95° C. for two hours, to permit oxidation of any vitamine *C* which might possibly be present, and was weighed out in equal amounts to each guinea pig. Four healthy young animals were chosen, three of them from the same litter, and the fourth of the same age and approximately the same weight. The following diets were employed:

A. Alfalfa meal + wheat flour, equal parts by weight, + 3 grams milk powder daily. Ground oats supplied freely.

B and *C*. Alfalfa meal + wheat flour, equal parts by weight, in the same amount as supplied to *A*. Three grams milk powder daily. About 20 to 30 grams lean beef daily.

D. Cracked oats in unlimited amounts. Three grams milk powder daily.

A and *B* were each given, in addition, about 10 grams of lactose daily, dissolved in water, and fed by pipette at each meal. McClendon[6] has shown that by feeding lactose the intestinal contents of rabbits may be changed from slightly alkaline to slightly acid, and in a later experiment (private communication) he obtained the same result with guinea pigs. The possibility exists that there is some relation between the large amount of vitamine required by the guinea pig and the alkalinity of its intestinal content.

Lactose, itself, does not possess any antiscorbutic value, as has been shown by Cohen and Mendel[7] and by Hart, Steenbock and Smith.[8] They explain its action in delaying the appearance of scurvy in an experiment by Pitz,[9] by suggesting that it may have carried some vitamine as an impurity. In the present experiment, the lactose was dissolved in water and boiled for six hours to destroy any vitamine that may have been present.

The weights and appearance of symptoms of scurvy of the guinea pigs are given in Table I. Both animals receiving meat outlived the controls, *B*, the one which was given lactose in addition, surviving *A* by seven days and

TABLE I

WEIGHTS OF GUINEA PIGS
(S = SCURVY)

DAYS	A	B	C	D
1	215	216	219	210
6	221	230	235	220
11	245	221	227	250
15	237S	233	243	267
16	240	244	244S	275
17	240	249	243	273S
22	238	256S	237	242
26	223	249	239	212
27	220	247	235	214
28	204	247	235	191
29	192	237	235	
30	170	232	232	
31		238	227	
32		235	214	
33		238		
34		230		
35		227		
36		224		
37		221		

surviving *D* by nine days. *C* survived *A* by only two days. *B* first showed symptoms of scurvy seven days later than *A*.

CONCLUSION

Meat may be fed to guinea pigs in considerable amounts, 20 to 30 grams daily, provided it is used fresh, and the guinea pigs are put on the diet when sufficiently young.

It possesses slight antiscorbutic value (sufficient to delay scurvy seven days). This has been shown by feeding it in combination with vitamine-free lactose which increases slightly the H-ion concentration of the intestine and thus may affect the stability of the vitamine present in the meat. This latter explanation is offered only as a suggestion.

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THE INFLUENCE OF FOCAL INFECTIONS IN DIABETES AS SHOWN BY ALTERATIONS OF THE BLOOD-SUGAR CURVE*

By Blair Holcomb, M.D., Portland, Ore.

THIS is a report of the histories of two patients with mild diabetes, which illustrates the effect of focal infection on the glucose tolerance and rather supports the theory of the infectious cause of diabetes.

Medical literature contains no reference to comparative blood-sugar curves before and after the removal of foci of infection in diabetic individuals. The lowering of tolerance by acute infections, such as measles, furunculosis, dental abscesses, etc., in an established case of diabetes is well known. Sansum[1] noted the decrease in tolerance in a normal person with acute tonsillitis. The rôle which influenza may have played in producing degenerative islet changes cannot be proved, but clinical evidence points strongly toward it as one of the major infectious causes following the late epidemic. Working with a large series of arthritides, Pemberton,[2] using the Janney[3] glucose meal, noted sustained elevations of the blood sugar in active joint infections and an abrupt return to a normal response following the removal of focal infections. Lusk,[4] quoting Rosenthal, states that diphtheria toxin given to rabbits renders the liver incapable of retaining glycogen and a hyperglycemia occurs. Olmstead[5] was unable to duplicate in arthritis the high curves of Pemberton, except with those patients who were febrile. Complete recovery of glucose tolerance, proved by blood-sugar curves, has not been reported, though Rohdenburg's[6] case of spontaneous disappearance of glycosuria at the menopause in a previously proved diabetic patient, showed hypertrophic pancreatic islets and an atrophy of the thyroid. The influence of infection was not considered nor were blood-sugar curves determined.

The mechanism by which toxins may influence the height of the blood-sugar curve is outlined by Olmstead and Gay.[7] They state in part: "1. Toxins may directly stimulate the action of liver and muscle diastase or inhibit the glycogenic power, or possibly disturb the hepatic acid base balance. 2. Focal toxins may act on glycogenolysis through their effect on the suprarenal medulla directly or through the autonomic reflex. 3. Focal toxins may also act on higher cerebral centers." With the discovery of the specific substance, insulin, the last link in the chain of evidence is complete. One may add, then, that most important of all may be the inhibitive effect of focal toxins or bacteria on the islands of Langerhans.

Mr. S., aged forty-two years, an American merchant, was seen June 5, 1923, because of a glycosuria that was discovered one week previously. Polyuria, polydipsia, and weakness began three months before, and a total loss of fifteen pounds of weight had occurred. His

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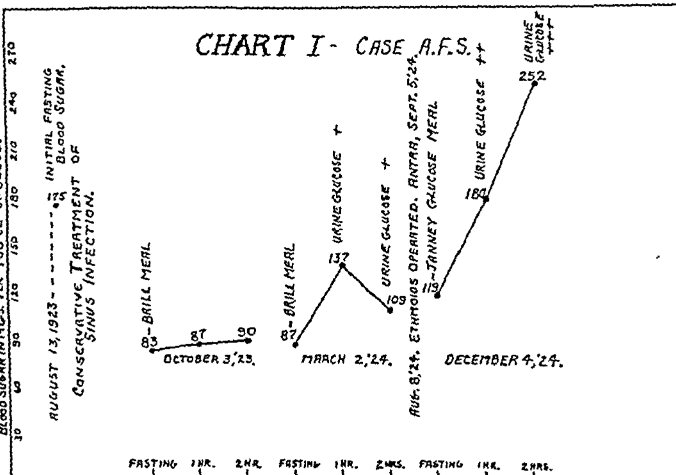
past history was negative except for jaundice with fever, pain, nausea and vomiting in 1908, but with no subsequent symptoms of gall bladder disease, scarlet fever as a child, and much nose trouble extending back for at least ten years. The latter consisted of mucopus drainage into the nasopharynx, many colds and finally an almost total obstruction of the nasal passages, so that at four different times during the ten years polyps had been removed, the last time in 1920. Physical examination showed an undernourished man 5 feet, 9 inches tall, weighing 138 pounds. The positive physical findings were one devitalized tooth, crepitant and mucous râles in both bases of the lungs, moderately enlarged thyroid (small colloid adenoma), and a blood pressure of 135-88. Nasal examination showed a bilateral hyperplastic ethmoiditis and purulent antra. Glucose was found in both morning and evening urines. The white blood cells numbered 13,500; the differential count, red blood cells and hemoglobin were normal. The stomach chemistry showed 12 free acid and 20 total acidity. The blood



Wassermann was negative. Gastrointestinal roentgenologic study gave no evidence of abnormality. Stereoscopic chest films revealed a rather pronounced bilateral peribronchial thickening and increased hilus shadows. The fasting blood sugar was 175 mg. per 100 c.c. of blood. A diagnosis of diabetes mellitus, chronic sinusitis and bronchitis was made and the patient was placed in the hospital. On a 91-G* the twenty-four-hour urine was negative the following day to Benedict's reagent, and the day following the blood sugar was 104 mg. The diet was rapidly increased to a G of 125, following which the blood sugar remained 104 mg. Subsequent diet adjustment carried the patient's G to 172, which raised his blood sugar to 117 mg. Reference to Chart I will show the details of the blood-sugar curves.

During this time the patient's antra had been washed several times. His cough and sputum had stopped, he had gained in weight to 141 pounds, and felt very well. He went home on June 29 on a diet G of 172 gm. On July 6 his blood sugar was 92 mg., and both antra were clear. On August 14 the blood sugar being 83 mg., his G was raised to 218.

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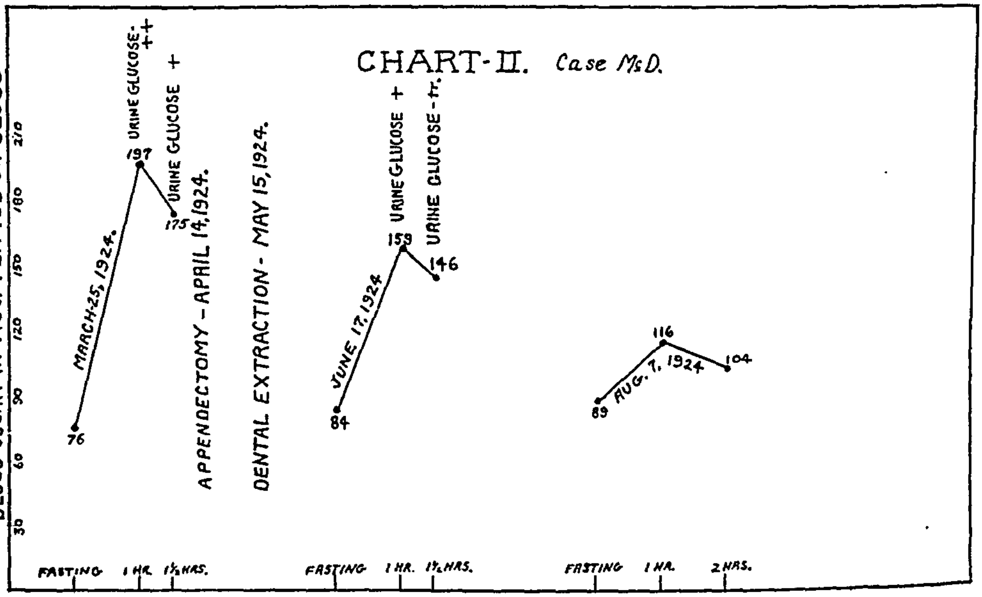
*G refers to grams of glucose in the diet and is calculated according to Woodyatt[1] from 100 per cent of the carbohydrate, 58 per cent of the protein and 10 per cent of the fat. The fatty acid glucose ratio of these diets, with the exceptions of diets above a G of 150 is 1.4. A diet of 1500 calories contains 91 G; 2000 calories 125 G; 2600 calories 150 G. Diets higher than these have an FA/G of about 1.1.

September 1 his blood sugar had dropped to 72 mg. On October 3, using the Brill[9] test breakfast, a blood-sugar curve was determined. The fasting blood sugar was 83 mg.; at one hour, 87 mg.; and two hours, 90 mg. We considered this a normal curve and instructed the patient to go onto a normal diet, restricting him only in the use of sugar. October 8 his fasting blood sugar was 92 mg. Reference to Chart I will show the details of the blood-sugar curves. On March 2, 1924, the blood-sugar curve was 87 mg. fasting; 137 mg. at one hour, and 109 mg. at two hours. Urine glucose by Benedict's reagent was present at the two latter times. On the basis of this low threshold alone, a diagnosis of renal glycosuria might have been justifiable, but knowing the previous history, it was untenable.

He did not report again until March 13, 1924. At this time, despite his statement that sugar had been found in the urine during the past three weeks, his fasting blood sugar was 82 mg. He was recovering from an acute cold of six weeks' duration during which his nasal discharge had increased. His diet was again restricted to a G of 250. The sinus infection was still active and subsequent conservative treatment was without results. The ethmoids



were operated upon August 8 and the antra later. Following this the antra were repeatedly clean and the nasal secretions were much decreased.

We did not see the patient again until December, 1924. Using the Janney glucose meal and giving 1.7 gm. glucose per kg. of body weight, the following curve resulted: fasting, 119 mg.; one hour, 180 mg.; two hours, 252 mg.; with sugar in the urine at one and two hours. Aside from the initial blood sugar the last fasting sugar was the highest he had ever shown. The diabetes is still of an essentially mild type, the patient's urine remains sugar free on a G of 250, and he holds a weight of 156 pounds and feels very well. Occasionally he experimentally raises his G to 275. This always causes glycosuria. The G of the Brill meal averages about 120 grams. This would amount to 360 grams for three meals. The first blood-sugar curve showed a definite normal response to this, so it is evident that he metabolized at least 360 grams at that time. Subsequently, despite the radical removal of pus, a loss of tolerance occurred.

Mr. McD., aged forty-three years, an American grocer, was seen March 18, 1924. Glycosuria was discovered during the course of a general examination. He had been suffering from cough, pain in the chest, in the back through the right sacroiliac region and down the right leg, and from polyuria for ten days following the removal of several abscessed teeth. He had previously had periodic attacks of pain in the right side of the abdomen for eight to

ten years, and pneumonia five years before with influenza. There was no history of diabetes in the family, no obesity, and no overeating. His weight was 187 pounds and height 5 feet, 8½ inches.

The physical findings were negative except for marked dental sepsis and tenderness along the cecum. The leucocyte count was 12,250 with 70 per cent polymorphonuclear neutrophiles. The blood Wassermann was negative. Both morning and evening samples of urine contained glucose with Benedict's reagent. The roentgenologic study of the gastrointestinal tract revealed a tender appendix with a broken lumen.

A blood-sugar curve, on March 25, using the Brill breakfast, showed a fasting level of 76 mg.; 197 mg. at one hour, and 175 mg. and one and one-half hours, with glucose in measurable amounts in the two latter urines. (Reference to Chart II will give the details.) The urine was rapidly desugarized on a G of 91 grams. The patient was then quickly raised to a G of 151 grams, on which his fasting blood-sugar level was 82 mg. On April 14 his appendix was removed. It was thick-walled, and the tip was swollen and acutely inflamed. After an uneventful recovery his blood sugar was 89 mg. fasting. May 15 all devitalized teeth were extracted. June 17 his blood-sugar curve had lowered to 84 mg. fasting; 159 mg. at one hour, and 146 mg. at two hours, with a measurable amount of urine glucose at one hour and a trace at one and one-half hours.

Because the patient wished to return to his work in the woods, where a measured diet would be impossible, an attempt was made to measure his tolerance. His G was raised to 198 on June 19. The blood sugar two weeks later was 92 mg. The G was raised subsequently by adding white bread and potatoes, until on August 5 it was 300 grams. The blood sugar following the raise in diet was 104 mg. fasting. A third blood-sugar curve on August 7 was 89 mg. fasting; 116 mg. at one hour, and 104 mg. at two hours, with no sugar in the urine.

An opinion was given that the tolerance had reached practically the normal limit; the patient was told to return to a normal diet, and was only moderately restricted in the use of sugar. Three months later the patient reported by letter that his blood sugar was 106 mg. fasting.

DISCUSSION

The above histories are significant because no evidence of obesity, overeating, or record of previous diabetes in the families could be obtained. They are further significant in that in both there was a clear-cut history of frank infection preceding the onset of diabetic symptoms. In Mr. S. the sinus infection had existed for at least ten years and there had been no acute exacerbation preceding the glycosuria, though later an acute cold had definitely lowered the tolerance. Washing the antra always had the opposite effect. Operating upon the ethmoids and antra did not raise the tolerance, but unquestionably removed the cause and cured the bronchitis. One might speculate that, had the nasal infection not been controlled, a more rapid progressive loss of glucose tolerance might have resulted. The final blood-sugar curve in this case shows an obvious diabetic range.

The removal of dental foci of infection is so definitely associated with the onset of diabetic symptoms in Mr. McD. that its influence in lowering the glucose tolerance cannot be denied. The effect of the appendectomy and the further dental extraction on the tolerance increase is debatable, as one frequently notes an almost unbelievable increase in the amount of glucose metabolized in mild diabetes by simply keeping the diet G well within the tolerance limit. On the contrary, many so-called refractory cases have yielded to the removal of focal infection. On the basis alone of the final blood-sugar

curve complete recovery of tolerance has apparently occurred. The objection may be raised, however, that because the Brill meal contains food which is absorbed over a longer time than the Janney glucose meal that it is not a sufficiently delicate test for mild diabetes. Brill, however, reported practically parallel curves in comparing the Janney meal with his breakfast in normal people and in mild diabetics.

(The blood-sugar methods used in this work were first, the Shaffer-Hartman[10] and later the Haskins-Holbrook[11] modification of the same.)

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LABORATORY METHODS

A GLASS DISC AS A STANDARD FOR THE DETERMINATION OF THE ICTERUS INDEX OF BLOOD SERUM*

By Marjorie Preston, A.B.

THE determination of the icterus index of the blood serum has been shown by Bernheim[1] and by Shattuck, Browne and Preston[2] to be of practical value to the clinician in ascertaining the accumulation of bilirubin in the blood.

The technic of the test, as described by Bernheim[1] is as follows: About 5 c.c. of blood are withdrawn from an arm vein and allowed to clot. After standing for a sufficient length of time, the blood is centrifuged until the serum is absolutely clear. Care must be taken to avoid hemolysis. The clear serum is then matched against a 1:10,000 solution of potassium dichromate (0.1 grams in 1,000 c.c. distilled water) which has been arbitrarily chosen as a standard solution. In our determinations the biocolorimeter,[3] an improved colorimeter of the plunger type has been used. The standard solution is placed in the left hand cup which is set at 15 mm. The serum is then placed in the right hand cup and matched against the standard. The scale reading is noted. The depth of the standard, divided by the scale reading of the unknown serum, gives the icterus index. For example: The scale reading of the serum is 3: the standard (15) divided by the scale reading of the serum (3) gives a quotient of 5, which is the icterus index.

In hyperbilirubinemia, the color of the serum may be too intense for a satisfactory comparison with the standard. In such cases the blood serum is diluted with physiologic salt solution (0.9 per cent sodium chloride solution) until it has roughly the same intensity of color as the standard. The unknown is then compared in the colorimeter with the standard. The icterus index is then corrected for the dilution of the serum. For example: if the blood serum were diluted ten times, and then read 3 against the standard at 15, the calculation would be $\frac{15}{3} \times 10 = 50$.

To obtain accurate and comparable results the potassium dichromate used should be Kahlbaum's *zur analyse* and should be weighed out on an analytic balance. The standard, when not in use, is kept in the dark and, to prevent a reduction to chromate, a few drops of concentrated sulphuric acid are added per 500 c.c. It was thought that the replacement of this standard by a glass disc would obviate the difficulties in the preparation and keeping

*From the Department of the Laboratories of the New York Post-Graduate Medical School and Hospital.

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of the dichromate standard. The Klett Manufacturing Company has prepared a disc of "Uran" glass which compares favorably with the potassium dichromate standard. The disc is 5 mm. in depth and is made with a flange which enables it to fit in the top of the plunger of the biocolorimeter. The plunger screws in as before but the cup is not used on the standard side. The determination is carried out exactly as described above except that the glass disc takes the place of the potassium dichromate standard solution. The color of the disc corresponds to the color of the standard solution set at 15 mm., making the calculation using the disc identical with that using the dichromate solution.

TABLE I

	DISC		DICHROMATE STANDARD	
PATIENT	READING	ICTERUS INDEX	READING	ICTERUS INDEX
De A.	2.6	5.7	2.6	5.7
A. T.	2.2	6.8	2.2	6.8
J. K.	4.4–4.5	17.0–16.5	4.4	17.0
F. P.	4.6	16.3	4.6	16.3
C. A.	2.4	31.0	2.4	31.0
F. P.	1.8	8.3	1.8	8.3
N. M.	4.5–4.6	16.5–16.3	4.6	16.3
E. G.	3.2	4.7	3.2	4.7
G.	4.2–4.3	3.4–3.5	4.2	3.5
C. A.	1.5	50.0	1.5–1.4+	50.0–53.5
F. M.	3.5	4.2	3.3	4.5
Y. K.	6.2	12.1	6.1	12.2
J. W.	1.3	11.5	1.3	11.5
J. R.	0.9	16.6	0.9	16.6
M. K.	1.7	8.8	1.6	9.3
S. M.	1.0	15.0	1.0	15.0
M. F.	1.1	13.6	1.0	15.0
J. G.	5.2	144.0	5.2	144.0

In a series of 18 comparisons representing a wide range of indices, sera were matched with both the disc and the standard solution. The results are given in the accompanying table. It is seen that in 14 out of 18 comparisons, the readings with the disc and the potassium dichromate solution were identical. The other four show a variation of 0.1 in three of the cases and 0.2 in one. Although the match was not always perfect, the color of normal as well as pathologic sera varies to such an extent that a standard which would give a perfect match in every case would be impossible to find.

SUMMARY

Nine months' experience with the disc standard in daily determinations of icterus indices has proved the disc to be as satisfactory as the dichromate standard for comparison with blood sera, and more convenient for use.

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THE TECHNIC OF THE PATHOGEN-SELECTIVE METHOD OF CULTURE*

By Myer Solis-Cohen, A.B., M.D., and A. I. Rubenstone, M.D.,
Philadelphia, Pa.

The object of the pathogen-selective method of culture is to separate those microorganisms from the surface or interior of the patient's tissues which are probably pathogenic for him, and hence infecting him, from those which are probably nonpathogenic for him, of which he is merely a carrier. The attempted differentiation is made *in vitro,* by means of the patient's fresh, whole, coagulable blood, which is believed to possess bactericidal or bacteriostatic power against organisms that are nonpathogenic for him and to lack it against organisms that are pathogenic for him. This inference is based upon certain experimental work done on both men and animals.

THE BACTERICIDAL OR BACTERIOSTATIC POWER OF WHOLE, COAGULABLE BLOOD

Wright[1] observed that blood as it issues from the capillaries has a bactericidal action upon pneumococci which is not found in defibrinated or citrated blood or in serum. Heist, Solomon Solis-Cohen and Myer Solis-Cohen,[2] seeking an explanation for natural immunity, found that if pneumococci are seeded *in vitro* in the fresh, whole, coagulable blood of the naturally susceptible mouse or rabbit, they multiply with great rapidity; while when similarly seeded in the blood of the naturally immune chicken or pigeon, they disappear. But they found that defibrinated chicken blood and pigeon serum exerted no bactericidal action upon pneumococci, this latter fact already having been shown by Strouse[3] and Kyes.[4] Lister[5] found that immunity to pneumococcus infection was not due to agglutinins and opsonins, while complement fixation has proved of little value in connection with the pneumococcus. Bull and Bartual,[6] using a different method of immunization from that employed by Heist and Solomon Solis-Cohen, found the whole, coagulable blood of the pigeon to inhibit the growth of pneumococci, which property was absent from mouse or rabbit blood, but stated that they found no proof of a true bactericidal action. Later (personal communication) Bull stated that he had been convinced that the whole, fresh blood of resistant animals may kill a certain number of pneumococci, having gained this impression from the fact that capillary tubes charged with high dilutions of culture and then filled with blood *failed to show growth after any length of incubation period,* but he still felt that it was impossible to say definitely whether this result is due to actual killing of the organisms or to an inhibi-

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tion of multiplication and spontaneous death. The real point of Heist and the Solis-Cohens' contention, however, and Bull now concedes it, is this: given a certain relation between the *number* of pneumococci (or other organisms) and the *volume* of blood, the organisms perish in the blood of the immune animal.

Smiley[7] found that the capillary tube (Heist-Lacy) test of Heist and the Solis-Cohens failed to distinguish between bactericidal and bacteriostatic power. He devised a test tube method which gave constant and conclusive results in indicating the bactericidal property of the blood of rabbits immunized against pneumococci, by which he demonstrated bactericidins in the blood of rabbits following the injection of pneumococci. The fact that the titer of bactericidal activity increases in proportion to the amount of pneumococci introduced suggests, according to Smiley, that there may be some relation between the bactericidal property of blood and active immunity.

Robertson and Sia,[8] who used a still different and quite elaborate method, state that their results indicate that the blood of resistant animals, at least of the dog and cat, possesses destructive properties for pneumococci, and that this destructive property is not possessed by the blood of certain susceptible animals. Their experiments suggest that natural immunity depends chiefly, if not entirely, upon this property. The leucocytes, they find, play an active part in this process, but whether or not the destruction of the pneumococci occurs entirely within the leucocytes is not determined. That the serum also plays a part is shown by the fact that when the serum of resistant animals was inactivated before being used in the serum-leucocyte mixture, the growth of even very small numbers of pneumococci was not prevented.

Matsunami and Kolmer,[9] using the method of Heist and the Solis-Cohens, incubated meningococci in the whole blood of rabbits, which are immune from meningococcic infection, and of mice, which are fairly susceptible. In rabbit blood little or no growth of meningococci had taken place after twenty-four hours' incubation, but in the blood of the mouse growth was vigorous. Matsunami[10] states in a later communication that he has found evidence that the action of the immune blood on meningococci is truly bactericidal.

Black, Fowler and Pierce,[11] who seeded with undiluted virulent cultures of the typhoid bacillus and of *Bacillus dysenteriae* the blood of rabbits immunized against these organisms and incubated the tubes for different periods of time, found that the organisms were destroyed at the end of five minutes, no bacilli or recognizable fragments being seen in the leucocytes. As the result of comparative studies with various antibodies they conclude that the bactericidal power of blood, thus determined, "is the most dependable criterion of the actual immunity of the animal."

According to Kolmer and Borow,[12] the Heist-Lacy method is a simple method for determining the bactericidal activity of whole blood at intervals after the administration of a drug and yields approximate quantitative results.

EXPERIMENTS ON MEN

In order to see whether or not bacteria known to be commonly harmful to men would grow more readily in human blood than those which are known to be practically harmless under ordinary conditions, Heist, Solomon Solis-Cohen and Myer Solis-Cohen[13] planted a strain of colon bacilli, isolated from feces, in the whole blood of fifteen healthy men, and a hemolytic streptococcus, isolated from a severe leg ulcer, following a shell wound, in the blood of sixteen healthy men. Almost no growth of colon bacilli had occurred after twenty-four hours' incubation, while vigorous growth of streptococci occurred in all but two of the sixteen human bloods tested. These two experiments they regarded as sufficient to prove that what had been found to be true of animals is also true of man: namely, that there is a certain correlation between the ability of bacteria to grow in the fresh, whole, coagulable blood of the individual or species tested and the virulence of the bacteria for that individual or species.

Myer Solis-Cohen and Heist,[14] in studying individual susceptibility and resistance, worked with many different microorganisms and different individuals. They found great differences in individual responses, indicating either variations in the pathogenicity of various strains of the same microorganism or variations in the personal resistance to a given strain of different human beings. We, therefore, believe it reasonable to infer that the fresh, whole, coagulable blood of any individual will destroy or inhibit the growth of organisms that are nonpathogenic for that individual, while it exhibits no such action upon organisms that are pathogenic for him.

The clinical results of Myer Solis-Cohen[15, 16, 17] and others[18] in cases of inoculation with killed bacteria, the vaccines consisting chiefly of organisms against which the patient's blood showed little or no antibacterial power, also seem to bear out the inference that growth of a given organism in the whole, coagulable blood of an individual indicates susceptibility to that organism, while failure to grow indicates resistance. That bactericidal or bacteriostatic power can be developed by means of inoculation with killed bacteria has been demonstrated experimentally by Solomon Solis-Cohen and Heist,[19] Bull and Bartual,[6] Smiley,[7] Robertson and Sia,[8] Matsunami,[10] and Black, Fowler and Pierce.[11]

DEFICIENCIES IN THE ORDINARY CULTURE

Bacterial cultures as ordinarily made (with the exception, of course, of those taken from the patient's blood) yield no information as to whether or not the organisms found are really infective. Quite frequently the predominating organism in such a culture is nonpathogenic for the patient, as indicated by the fact that it disappears when grown in his whole blood. Yet most bacteriologists regard the predominating organism as the etiologic factor in an infection and prepare their vaccines accordingly. Consequently, such vaccines at times probably have little or no specific action, whatever may be their value as indifferent proteins.

The ordinary culture may sometimes fail to reveal the etiologic organ-

ism, especially when the latter occurs in such small numbers that it is overgrown or has its growth inhibited by other organisms which are present in larger numbers, but are nonpathogenic for the patient. The latter may, indeed, be of pathogenic varieties; but the point is that, at the moment, they are not pathogenic for the person harboring them, who is a carrier only. Even should a vaccine made in the ordinary way contain among the nonpathogenic varieties a few of the etiologic organisms, the vaccine can have but a minimal specific value, since the dose of the specific antigen bears such a small proportion to the dose of vaccine given as to be practically negligible.

TEST FOR SPECIFIC PATHOGENICITY

In the pathogen-selective culture, however, the predominating organisms that are nonpathogenic for the patient are destroyed or have their growth inhibited, thus permitting a relatively free growth and multiplication of the organisms that are pathogenic for the patient, even if on the original smears they were proportionately few in number. In other words, even though the whole blood should fail to kill those organisms that are not pathogenic for him, it retards their multiplication, while exaggerating the growth of those organisms that are pathogenic for him. So that on smears from cultures made in whole blood and then recultured on a plain culture medium, the predominating organisms are regarded as pathogenic for the patient, while those found in a small proportion are regarded as relatively harmless for him. This method not only has the advantages of simplicity and promptitude over mixed culture passage through animals in picking out organisms but is also more definitely specific because the organisms are brought in direct contact with the patient's own blood.

The method, of course, has limitations. The reaction is, to a certain extent, quantitative, and is thus affected by the relative proportion of blood and bacteria engaged. When a relatively large number of organisms, nonpathogenic to the patient, are planted in a relatively small quantity of blood, it is conceivable that the amount of bactericidal or bacteriostatic substance present may not be sufficient to destroy or inhibit the growth of all of them; and, if only a few escape, they may multiply rapidly in the absence of any restraining influence. Conversely, if relatively few organisms are planted in a relatively large quantity of blood that is but slightly inimical to them, it is possible that enough of the restraining substance might be present to prevent their growth entirely.

THE PATHOGEN-SELECTIVE CULTURE

The procedure in obtaining cultures from body recesses, fluids and excretions, is as follows:

For each culture the materials necessary are:

1. One culture tube, containing preferably a rich medium such as Löffler's serum agar.
2. One empty sterile test tube (small).
3. One sterile cotton-tipped applicator.
4. Sterile syringe and needle (gauge 19-23).
5. A Bunsen or alcohol flame.

The *primary* culture is obtained with sterile applicator and inoculated in a tube of rich culture medium. (This is called the plain culture.) The same applicator is then introduced into the sterile test tube and a very thin film is inoculated on the bottom and side of the tube. Blood from the patient is immediately obtained by venipuncture and about 3 to 5 c.c. are allowed to run into the empty test tube recently inoculated. (This is known as the blood culture.) Where multiple recesses or fluids are to be cultured the procedure is repeated. All are, of course, done simultaneously so that the requisite amount of blood can be obtained by a single venipuncture and distributed *to all the empty inoculated tubes at once. Both the plain and blood* tubes are then incubated for twenty-four hours, and then the plain cultures are placed in an ice chest to prevent further development. The clotted blood is then removed from the blood tube and, with a sterile platinum loop, the residual blood at the bottom of the tube is inoculated on one or more tubes of plain culture medium in a thin film on the surface and is incubated for twenty-four hours.

At the end of that period the plain cultures previously placed in the ice box and those inoculated from the blood tubes are examined for organisms. Frequently one can at a glance differentiate types of organisms such as streptococci, etc., because of the blood film on the plain medium.

PREPARATION OF VACCINE

In the preparation of vaccine, subculture should be avoided in order that relative proportions of growth be maintained. The culture tubes are filled to the top of the slants with sterile normal saline solution; the growths are washed off with the aid of a platinum loop and agitation, and the suspension of vaccine is made so that the organisms that grow out on the blood-tube cultures are in a 9:1 proportion to those that are present only on plain cultures.

The vaccine, therefore, will consist chiefly of those organisms that grew out in spite of contact with the patient's blood, with the addition of 10 per cent of organisms that grew up on the plain culture but failed to grow up in the patient's whole blood, so that any possible antigenic effect of symbiosis is preserved.

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DISCUSSION

Dr. Herman Spitz.—I would like to report a little modification of this method that we have been using in gonorrhea cases. We place two or three cubic centimeters of the patient's whole blood on agar slants and then inoculate them with the pus or prostatic secretion. We then remove all growth, wash it, and prepare the vaccine from the washed sediment. We have found that many of the organisms do not grow at all. In giving the vaccine we always advise the physician to use large doses and to get a reaction, not only local but systemic. I feel that unless we get a very good reaction the vaccine is practically useless. The vaccine has been productive of good results by this method in a fair number of chronic infections.

Dr. O. Lowy.—I would like to ask Dr. Rubenstone whether he has done any complement fixation in connection with his work. I have been very interested in that, especially in arthritis.

Dr. Myer Solis-Cohen (closing).—You must distinguish between the organisms that are infecting the patient and other organisms present. The purpose of the pathogen-selective culture is to inhibit the growth of or to destroy the organisms that are nonpathogenic for that particular patient and to encourage the growth of organisms that are pathogenic for him. This method has been used in arthritis, asthma, endocarditis and in various other conditions, in every condition where there is an infection. The surgeon thinks he has removed the infection when he merely removed the infected tissue. The bacteriologist frequently thinks that he has prepared effective vaccine, when in reality the etiologic organism may never have appeared in his culture, which may have contained only organisms that were nonpathogenic for the particular patient. As regards the administration of vaccine, it may be that in gonorrhea it is all right to give very large doses, but in most other conditions a really specific vaccine must be given very cautiously, according to the reaction from the preceding dose.

PERMANENT STANDARDS TO BE USED WITH BENEDICT'S "CLINICAL QUANTITATIVE TEST" FOR SUGAR IN URINE*

By Jeanette Allen Behre, Ph.D., and William Muhlberg, M.D., Cincinnati, Ohio

ABOUT two years ago Benedict[1] described a "clinical quantitative test" for sugar in urine. At the same time Folin[2] published a "clinical quantitative test" for albumin in urine. These tests have apparently not been published in any scientific journal and so may have escaped the attention of many physicians and laboratory workers who would find them useful. The main object of the new procedures was to provide very simple and relatively accurate methods for the quantitative estimation of small quantities of sugar and of albumin, such as may be responsible for the so-called "borderline" reactions for these substances in urine. Thus the new tests make it possible to record in definite figures the quantities of sugar or of albumin which have heretofore been recorded as "trace," "slight trace," etc., and so to decide upon a more definite basis whether or not the quantities found indicate a pathologic condition. Using these tests it is also possible to follow with accuracy the progress of the patient showing these "borderline" conditions. The sugar test has been used with highly satisfactory results in the laboratories of many of the insurance companies and it would seem to be very practical for use in a wide number of instances where routine examinations are being made, such as in hospitals, private laboratories, etc.

It is the object of the present paper to report the preparation of permanent standard solutions for use in the Benedict "clinical quantitative test" for sugar. Before presenting these findings we feel that it will be of service to present briefly the original directions for the method.

The determination is carried out in test tubes of equal diameter, graduated to 25 c.c. One c.c. of urine, 3 c.c. of 0.2 per cent picric acid and 0.5 c.c. of 50 per cent acetone (prepared fresh each day by dilution of acetone) are added in the order named, and the tube transferred at once to a boiling water-bath and heated for from ten to fifteen minutes, cooled and the contents diluted with water to 25 c.c. As many as ten determinations can be made at one operation. The color is then compared in a side-by-side inspection, by transmitted light, with the color of a set of standards contained in tubes of the same diameter. Benedict recommends that quantities of sugar up to 0.2 per cent, in urines of low specific gravity, or of 0.3 per cent, in urines of high specific gravity (over 1.025), should be considered as normal.

The standards described in the original directions are made by dilution of an alkaline picric and picramic acid solution, and they maintain their color for

*From the Laboratory of the Union Central Life Insurance Co., Cincinnati, Ohio.
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only about a month, which is an inconvenience in routine work. The standards described in the present paper are made of potassium bichromate, chromium potassium sulphate ("chrome alum") and sulphuric acid, and the combination has been found to be perfectly stable when kept in sealed tubes. We have, in this laboratory, tubes which have been kept for a year, much of the time in bright sunlight, and which show no change from the original color.

The color matches that given by glucose solutions, and by urinary sugar, almost perfectly, not only in a side-by-side comparison (by transmitted light), but also when the reading is made in a colorimeter.

The relative amounts of the constituents used vary in the different standards, the formula for each standard having been worked out arbitrarily so that it would most nearly match the color given by a pure glucose solution of the given concentration. For the 0.1 and 0.2 per cent standards it was found that the addition of some cobalt chloride made the reading better in the colorimeter.

The standards are put into tubes of the same diameter as those used in the sugar determination. These tubes are first drawn out at the end, the liquid is introduced through the small opening, and the tubes sealed off so that they are perfectly air-tight. If exposed to the air, the solutions darken.

The directions for making the standards follow:

DIRECTIONS FOR MAKING STANDARDS

(Chemically pure compounds should be used)

0.5 per cent Standard.—8 gm. potassium bichromate dissolved in water with 6 c.c. concentrated sulphuric acid. Cooled. 0.6 gm. chrome alum added and dissolved. Made up to 100 c.c. with distilled water.

0.4 per cent Standard.—6.66 gm. potassium bichromate dissolved in water with 5 c.c. concentrated sulphuric acid. Cooled. 0.55 gm. chrome alum added and dissolved. Made up to 100 c.c.

0.3 per cent Standard.—4.6 gm. potassium bichromate dissolved in water with 5 c.c. concentrated sulpuhuric acid. Cooled. 0.5 gm. chrome alum added and dissolved. Made up to 100 c.c.

0.2 per cent Standard.—0.52 gm. potassium bichromate dissolved in water with 1.5 c.c. concentrated sulphuric acid. Cooled. 0.1 gm. chrome alum and 3.2 gm. cobalt chloride added and dissolved. Made up to 100 c.c.

0.1 per cent Standard.—0.075 gm. potassium bichromate, dissolved in water with 1.5 c.c. concentrated sulphuric acid. Cooled. 0.172 gm. chrome alum and 1.48 gm. cobalt chloride added and dissolved. Made up to 100 c.c.

SUMMARY

Directions are given for the preparation of permanent standards for use in the Benedict "clinical quantitative test" for sugar in urine.

The original directions for Benedict's method are briefly presented.

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A NOTE UPON A SIMPLE METHOD OF QUANTITATIVE BLOOD CULTURE*

By Robert A. Kilduffe, M.D., Atlantic City, N. J.

THE use of bacteriostatic or bactericidal dye compounds in the treatment of bacteriemias has emphasized the value of determining at times the degree as well as the presence of bacterial blood infections through the enumeration of the bacteria in a known quantity of blood.

This procedure is readily and simply incorporated as a part of routine blood culture by the method in use in these laboratories and described below.

A 10 c.c. syringe is wrapped in paper or muslin, barrel and plunger separated, and sterilized by dry heat. The needle is placed in a stoppered small tube and sterilized separately.

Bacteriologic test tubes are plugged with cotton and sterilized and sufficient 2 per cent sodium citrate in normal saline is added to make a layer about one inch deep in the tube. In similar tubes exactly 2 c.c. of distilled water are placed and both sets are autoclaved.

The specimen is drawn by venipuncture after sterilization of the arm. A known volume, 1 or 2 c.c. or some other convenient quantity, not more than 5 c.c., is ejected into the distilled water tube. The remainder of the blood is ejected into the citrate tube.

On the label of the distilled water tube is noted the amount of blood used.

Both tubes are then brought to the laboratory, and inoculations are made at leisure, though the time interval between collection and inoculation is as short as possible.

Knowing the volume of water and blood, the volume representing one centimeter of blood is easily calculated, and this amount is transferred by a sterile pipette to a sterile Petri dish. Agar, 1.5 per cent, plain or glucose, melted and cooled to 40° C., is then poured, the blood and agar mixed, and the plate allowed to solidify.

From the citrate tube inoculation is made to a flask containing 100 c.c. of 1 per cent glucose broth.

After incubation the plate shows the number of colonies per cubic centimeter of blood, and the broth flask gives sufficient growth for all purposes.

The blood being laked, both depth and surface colonies may be counted.

For slow growing organisms, or where the number is few, growth may be evident in the flask before it is clearly seen on the plate. In such cases a preliminary report is made of the nature of the growth, and the numerical intensity of the infection is reported later.

One culture serves as a check on the other. Information, in positive cultures, is obtained simultaneously both as to the nature and to the intensity of infection.

*From the Laboratories of the Atlantic City Hospital.

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A MODIFICATION OF THE UNGER BLOOD TRANSFUSION APPARATUS*

By H. W. Jones, M.D., Philadelphia, Pa.

The Unger blood transfusion apparatus has been used successfully by us in some fifteen hundred transfusions. As originally described, its use requires the presence of two assistants, one to inject the normal saline solution and one to pour ether on the syringe by which the blood is transfused. The following modifications have been made and are herewith described:

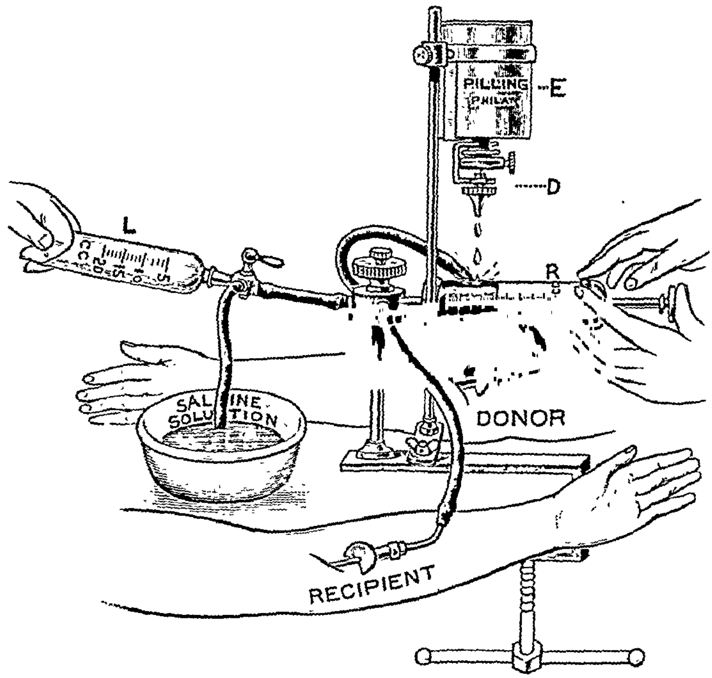

Fig. 1.—*E*, ether; *D*, dropper; *R*, Record syringe for blood; *L*, Luer syringe for salt solution.

1. An attachment, which holds the ether can so that a stream of ether of variable size and force is permitted to fall on the blood-bearing syringe, is shown in the plate. A needle valve, by which the size of the stream can be controlled, is inserted in the top of the can.

2. The caliber of the Unger needle is modified by enlarging the inside diameter. The shoulder is wider, making its position more secure.

3. The curve of the attachment that connects the rubber tubing to the donor's needle is made less. This eliminates a point of resistance with which the blood from the donor's needle comes into contact, thereby lessening the chance of coagulation at that point.

*Received for publication, February 17, 1926.

4. The bed of the screw fastening at the side which holds the inner portion of apparatus in place is made deeper. This prevents movement and displacement, and prevents the entrance of air.

5. A two way stopcock is added to facilitate the filling of the syringe with normal salt solution without disconnecting it.

6. The record syringe is held in place by a bayonet joint. This prevents the syringe from slipping, thereby eliminating another source of air entrance.

7. The bore of the steel connecting tubes to donor's needle and recipient's needle is increased. This eliminates further points with which the blood might meet with resistance in its transmission.

We feel that the ether attachment simplifies the operation because it does away with one assistant and because ether is not wasted unnecessarily. The old method of spraying the ether was most objectionable to the patient.

The other changes lessen the danger of air influx and help to prevent the formation of early products of coagulation.

A SIMPLE METHOD FOR FINDING DIBOTHRYOCEPHALUS LATUS EGGS IN THE STOOLS*

By Harry B. Schmidt, M.D., Detroit, Mich.

THERE are a number of complicated methods of examining stools for the eggs of the Dibothrycephalus tapeworm. The method which I have used for the past ten years has been very satisfactory and is time saving, when contrasted to other methods.

The procedure is as follows:

After the stool has been mixed with a small amount of normal saline solution and centrifuged, a small portion from the supernatant and bottom sediment is placed on a slide and a cover-glass is pressed down firmly with a blunt instrument. After some experience one can ascertain just the right amount of pressure to use in order to raise the caps on the eggs. Under the low power of the microscope, eggs with open caps are easily and quickly differentiated from other material in the stool sediment. By this method a slide per minute can be satisfactorily examined under the low power of the microscope.

In nine out of ten cases the stools do not have to be centrifuged, but simply mixed with water and examined as described.

*Received for publication, March 15, 1926

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

Maloney, P. J., and Weld, C. B.: Diphtheria Toxin and Antitoxin Flocculation, (Ramon Test). Jour. Path. and Bacteriol., Edinburgh, October, 1925, xxviii, 655.

The authors report their investigation of the test devised by Ramon (Ann. de l'Inst. Pasteur, 1925, xxxix, 1), which is based upon the flocculation occurring when suitable mixtures of diphtheria toxin and antitoxin are brought into contact.

The procedure consists of adding to constant amounts of diphtheria toxin (or toxoid), in a series of tubes, varying amounts of diphtheria antitoxin and incubating the tubes at a temperature not to exceed 50° C.

The first tube in which flocculation occurs is termed the "indicating tube" and, according to Ramon, contains a balanced mixture of toxin and antitoxin.

Ramon believes that this test may be used as an in vitro method of antitoxin assay and also as a measure of the relative immunizing power of toxoids.

Inasmuch as conflicting reports have been made by various observers, Maloney and Weld conducted tests upon trial bleedings from 31 horses under immunization. In all, 100 tests were made.

The strength of the antitoxin was estimated by Ramon's method and by the usual animal method for the standardization of antitoxin.

A perceptible divergence occurred in the results, deviations of 200 or more units being noted.

The exact nature of the Ramon test is as yet unexplained.

The authors believe that it has a field of usefulness, the extent of which, however, must be determined by further studies.

In about 80 per cent of antitoxic sera the flocculation unit value is about 20 per cent of the animal value. The test, therefore, may be used as a rough guide to the antitoxin unitage.

The occurrence of flocculation may also serve as an index to the antigenic value of the toxins or toxoids, as those which failed to produce flocculation also failed to produce immunity.

They conclude that the Ramon test cannot be used for the exact determination of the antitoxic value of an immune serum, or for reliable indication or representation of a balanced mixture of toxin and antitoxin.

Wright, H. D.: The Bacteriology of Acute Infectious Endocarditis. Jour. Path. and Bacteriol., Edinburgh, October, 1925, xxviii, 541.

The purpose of the investigation was to determine the frequency of streptococcemia in acute infectious endocarditis, in view of the number of cases reported in which sterile blood cultures have been obtained.

The isolation of streptococci from the blood stream was found to be a relatively simple procedure, a wide variety of culture media being suitable. The failure to obtain growth, therefore, and especially when the cultures were sterile on several attempts, might be taken as reliable evidence that organisms were not present in the sample examined and that a bacteriemia did not exist.

Minor variations in technic had no apparent influence on the frequency with which positive cultures occurred.

Either liquid or solid media was suitable. There was no advantage in the use of anaerobic methods.

Media containing trypsin or sodium citrate (not to exceed 0.2 per cent) were slightly superior. The addition of lactic acid was without advantage.

Streptococci may be detected by the usual methods in vogue when present in no greater numbers than 1 to 2 per c.c.

If the specimen is kept at room temperature delay in inoculation is without effect on the viability of the organisms.

Delay in the appearance of growth is due to a prolongation of the latent period and dependent upon peculiarities of the organism rather than upon inhibitory properties of the blood.

Agonal or postmortem cultures were unreliable because of the frequency with which streptococci are concerned in terminal invasions.

The frequency of bacteriemia in endocarditis varies. In some cases there are no bacteria found in the blood stream; in others they are liberated as secondary invaders from the valvular lesions. The blood may also be actively bactericidal. A negative culture, therefore, does not negative the diagnosis.

When the blood culture is positive, bacteria may readily be demonstrated on the valves, in sections. Where cultures are sterile bacteria may also be seen, but they appear to be disintegrated.

The author believes that the streptococcus is the agent of most importance in the causation of acute bacterial endocarditis.

Brown, J. H.: A Study of Anaerobic Bacteria. Jour. Bacteriol., November, 1925, x, No. 6, p. 513.

Brown calls attention to the fact that relatively detailed studies of anaerobes may be made with simple test tube methods, and he reports such studies.

He used, for the determination of various biologic characteristics, the simple methods mentioned below.

Cooked meat medium under vaselin was observed for changes in color, consistency, digestion, and gas formation. A black ring at the surface was taken as indicative of hydrogen sulphide formation.

Such cultures were also used for studies of morphology.

Deep agar shake tubes demonstrate the character of deep colonies and give information in a rough way as to the most favorable degree of anaerobiosis. Veal infusion gelatin is incubated at 38° C. and then placed in ice water. Skimmed milk under vaselin and citrated milk are observed for gas formation, coagulation, and peptonization.

Protein digestion is tested by placing 5 mm. cubes of coagulated horse serum, egg white, rennet casein, and fibrin in veal broth tubes which are then autoclaved.

Carbohydrates were sterilized in 10 per cent solution and added aseptically to tubes of sterile broth under vaselin, small bits of rabbit or guinea pig kidney being added to the broth tubes before autoclaving and prior to the addition of the sugar.

The addition of indicators was not found feasible and resort was had to P_H determinations as evidence of fermentation. The best evidence, however, was obtained by adding only 0.2 c.c. of the sugar and, after growth for three months, testing for the presence or absence of sugar by Benedict's test. If fermentation had occurred the sugar was destroyed and there was no reaction.

For mannite, glycerol, and salicin, the culture, after a month's incubation, is heated in a boiling water-bath for fifteen minutes and inoculated with B. coli. The absence of gas formation must be interpreted with caution because of the possible presence of thermostabile gas inhibiting factor; the presence of gas indicates that the anaerobe failed to destroy the small amount of sugar in the medium.

Hydrolysis of milk fat is a useful differentiating point.

Thick cow's cream is washed several times with normal saline and recovered by centrifugation. A thick emulsion is made with normal saline and autoclaved. About 0.5 c.c. is pipetted aseptically into 5 c.c. of broth under vaselin. In time the cream forms a compact

layer 1 to 2 mm. thick below the surface of the vaselin. Hydrolysis is indicated by the transformation of this layer into a thick layer of gray flocculent material composed largely of fatty acids and soaps.

Various chemical titrations may be included and are reported in the study.

The necessity for such studies of the anaerobe group is emphasized and the characteristics of a number of anaerobes so studied are reported. Certain "metabolic groupings" of anaerobes are described.

Dold, H.: Critical Comparison of the Determination of the Germ Content of Bacterial Vaccines. Deutsch. med. Wchnschr., November 6, 1925, li, 1851.

Dold discusses the accuracy of various methods which have been proposed for the numerical standardization of bacterial vaccines.

Wright's method he considers inaccurate because: (a) the number of red cells in the blood used for comparison varies; (b) some of the cells and bacteria may be lost in staining; (c) cells may be superimposed on bacteria and these be unnoticed; (d) bacteria may vary in visibility; (e) cellular débris and platelets may be confused with bacteria; and (f) the uncontrolled personal factor.

He believes a more accurate method to be that of Fries in which the bacterial suspension is compared with a standard suspension of yeast by a method similar to Wright's. Some of the criticisms applied to Wright's method apply also to this.

The most accurate method is direct count of the organism in a counting chamber, but this is not applicable to all organisms.

Indirect methods, such as weighing, centrifuging (Hopkins), and nephelometry are all used, but all possess inherent errors.

Inasmuch as the numerical concentration of the organisms does not necessarily indicate the antigenic value of the vaccine; since antigenic substances may be in solution, and since the efficiency of the vaccines depends upon various factors besides their numerical strength, Dold believes that the procedure is impracticable and without scientific basis.

Finerund, C. W.: Ulcus Acutum Vulvae (Lipschütz). Arch. f. Dermat. u. Syph., January, 1926, xiii, No. 1, p. 55.

In this case report attention is called to an acute ulceration of the vulva due to an organism originally called B. crassus but since shown to be identical with Döderlein's bacillus.

It is best grown aerobically on serum-sugar-stab cultures or maltose-ascitic-fluid-agar plates on which it appears as delicate hairy outrunners on the borders of abundant and varied growths.

Later it turns grayish-yellow. In older cultures long threads are seen. It is a rather thick, gram-positive bacillus.

The disease is frequently confused with other conditions.

The clinical picture is described in detail, and the paper is illustrated with microphotographs showing the organism and the lesions.

Schamberg, J. F., Harkins, M. J., and Brown, H.: Gold Compounds in Treatment of Experimental Tuberculosis of Skin in Animals. Arch. f. Dermat. u. Syph., January, 1926, xiii, No. 1, p. 43.

In an illustrated paper chemotherapeutic studies of a number of gold compounds in skin tuberculosis in rabbits are reported in detail.

The authors believe a favorable, though not curative, effect is produced by gold therapy and that the subject deserves extensive study.

The study was suggested by the work done abroad with "Sanocrysin."

The paper should be read in the original for the details which do not lend themselves readily to abstraction.

Jaffe, R. H.: Amyloidosis Produced by Protein Injections. Arch. Path. and Lab. Med., January, 1926, i, No. 1, p. 25.

The production of amyloidosis by repeated injections of nutrose is confirmed in white mice. While amyloidosis may occur in the spleen after 40 injections (daily) of 0.2 c.c. of a 3 per cent solution, 60 injections are required for its uniform production. Larger doses do not hasten its development. After 70 injections even the heart valves may be involved. If the injections are continued, an endothelial absorption takes place in the liver. Human or animal serum may be used with similar results.

Hypochromatic anemia and leucocytosis (polymorphonuclear) occur.

Amyloidosis results from acquired hypersensitiveness to the injected substance.

Two microphotographs and two colored plates accompany the paper.

Lang, F. J.: Role of Endothelium in the Production of Polyblasts (Mononuclear Wandering Cells) in Inflammation. Arch. Path. and Lab. Med., January, 1926, i, No. 1, p. 41.

An experimental study of the histogenesis of mononuclear giant cells is reported in detail with 10 illustrations.

The polyblasts arise partly through the mobilization of local histocytes, the resting wandering cells of the loose connective tissue, and partly through the rapid hypertrophy of emigrated lymphocytes and monocytes. The hematogenous cells quickly join with the histocytes in their transformation.

The endothelium does not give rise to ameboid elements but to fibroblasts. There is no production of "endothelial leucocytes."

LeCount, E. R., and Singer, A. H.: Fat Replacement of the Glycogen in the Liver as a Cause of Death. Arch. Path. and Lab. Med., January, 1926, i, No. 1, p. 84.

Attention is called to the fat replacement of the glycogen in the liver as the sole autopsy finding and as a cause of sudden death in alcoholics. Two autopsies are reported and eleven cases discussed.

Corper, H. J.: A Control Method of Staining Smears for Tubercle Bacilli. Arch. Path. and Lab. Med., January, 1926, i, No. 1, 93.

The necessity for controlling the technic is emphasized and a method suggested for this purpose.

A suspension containing 5 to 10 mg. of bacilli in 1 c.c. of equal parts of normal saline and acetone is prepared by grinding a weighed amount of bacilli in a centrifuge tube with a small glass rod, at first dry, then with saline drop by drop until one-half the total volume of the solution has been added. Then the acetone is added slowly with stirring.

The suspension is kept in a rubber stoppered bottle and shaken before use. It is used to make a control smear with the material to be examined. Due precautions must be taken to avoid accidental contamination of the specimen with the control material.

Both smears are then simultaneously stained in a specially constructed holder so that they are subjected to simultaneous procedures.

Wilson, W. F.: A Simple Apparatus for Obtaining Cultures of Anaerobes. Jour. Path. and Bacteriol., Edinburgh, October, 1925, xxviii, 673.

The apparatus presented is a newer form of that originally described by the author in 1917. It is made of porcelain, since the original, being made of tin, was gradually corroded by the action of the caustic soda and pyrogallic acid solution.

It consists of a hollow disc-like container with a central circular opening. At the sides are two nozzles *C* and *D*.

A few teaspoonfuls of dry pyrogallic acid are placed in the cavity of the disc. The inoculated Petri plates, which may be piled one on top of another, are placed on the shelf

made by the edges of the circular opening in the disc. They are then covered by an inverted beaker or jar which is sealed to the disc by plasticine.

C is connected to a vacuum pump; *D*, by means of pressure tubing, is connected through a glass T-tube to a gas tap and to a glass tube inserted into a jar containing the alkaline fluid described by Rockwell: acid sodium phosphate, 0.75 gm.; sodium bicarbonate, 50 gm.; water 500 c.c.

The tube at *D* is clamped and air drawn out of the apparatus. The clamp attached to the gas tap is then released and gas passed through the apparatus so that it fills and empties twice. A vacuum is then created and 20 c.c. of the alkaline fluid drawn into the main cavity of the disc, when the tubes leading from *C* and *D* are clamped off and the apparatus incubated.

Menninger, W. C., and Holder, E. H.: The Relation of Total and Polymorphonuclear Leucocyte Counts in Chronic Appendicitis. Ann. Surg., December, 1925, lxxxii, No. 6, p. 960.

The authors report a series of 93 cases of chronic appendicitis in which the diagnosis was corroborated by microscopic studies of sections.

The leucocytes were charted by the method devised by Gibson, which applies the observations made by Sondern in 1905 that the total white cell count may be taken as an index of the degree of bodily resistance and the polymorphonuclear count as indicative of the severity of the infection.

Taking 10,000 as the outside normal limit of the total white cell count, and 75 as the normal extreme of polymorphonuclear percentage, the total count is charted on the right side of the graph and the percentage count on the left. For each 1,000 increase in the total count there should be an increase of one per cent in the polymorphonuclears. When the increase is thus proportional a line drawn from one side of the chart to the other will approach the horizontal. A rising line indicates a proportionally poor resistance and vice versa.

The authors use the term ''resistance index'' to express the disproportion between these two factors. The results of their studies: a falling line in chronic appendicitis as opposed to a rising line—a poor resistance index—in acute appendicitis and general peritonitis.

Mock, H. E., and Ellis, J. D.: Trauma and Malignancy. Jour. Am. Med. Assn., January 23, 1925, lxxxvi, 257.

The possible relation of trauma to the production of neoplasms has become a matter of importance in connection with compensation cases. The authors endeavor to epitomize the available data concerning this question and suggest, as a result, that before it can be said positively that a malignant tumor is the direct result of a single trauma, the following postulates should be fulfilled:

1. Definite description by the reporting surgeon of the trauma at the time it was sustained.

2. Definite proof, by every possible means of examination made at the time the injury was sustained, that no tumor already existed at the site of trauma.

3. Definite signs and symptoms of a pathologic process, continuing at the site of the trauma until a malignant tumor appeared and was positively diagnosed.

Thus far, they have been unable to find a single case fulfilling these requirements.

To constitute reasonable proof of trauma sufficient to cause definite tissue changes they suggest:

1. Reasonable proof of a trauma of sufficient seriousness to cause definite tissue changes;

(a) The injury to be reported within a reasonable period after the accident.

(b) The examination and condition of the traumatized tissue to be reported by a physician within a reasonable period after the injury.

2. The developing neoplasm must be at the same site as the original injury and must involve some of the tissue which, without reasonable doubt, could have been involved in the original trauma.

3. Definite evidence must be produced to prove that no neoplasm existed at the site of injury prior to the accident.

4. In addition to the history of trauma there must be a history of definite bridging signs, such as a persistent swelling, an unhealed wound, or anatomic and functional disturbances which connect the trauma with the malignant growth; subject symptoms, such as complaint of pain, tenderness, or weakness, are impossible to evaluate or visualize and therefore must not be considered competent evidence.

5. The time that has elapsed between a given trauma and the development of the malignant tumor need not be considered if the foregoing conditions have been present; however, it is safe to say that a malignant tumor that develops within two weeks after the trauma existed prior to the injury.

6. A preexistant malignant tumor may be aggravated or accelerated in its growth by a trauma; the trauma may be the first factor to call the patient's attention to the tumor.

7. In the case of metastatic or secondary tumor developing at the site of trauma, the first four postulates must be met before the employer is held responsible, and such responsibility should be limited to the treatment of the local condition: it should not be extended to include responsibility for the death that is bound to occur shortly, as the trauma could not have aggravated the primary growth.

Illustrative cases are cited in which the relation between trauma and neoplasm was thus reasonably indicated and which were considered compensable.

Grott, J. W.: The Titration of Gerhardt's Reaction in the Urine in Comatose Conditions. Presse méd., Paris, November 14, 1925, xxxiii, 1507.

Quantitative determination of acetone in the urine is a tedious and complicated procedure. The author, therefore, makes use of Gerhardt's reaction for aceto-acetic acid, to which, by the procedure described below, he has endeavored to impart a quantitative factor. This test becomes positive, according to Grott, when the excretion of acetone and aceto-acetic acid reaches four grams daily.

To 10 c.c. of urine add 10 drops of iron perchloride solution and titrate with N/10 sulphuric acid until the color is cherry red. A 1:2000 solution of antipyrin is used as a color standard.

The amount of acid required is calculated for 100 c.c. of urine and is termed the concentration factor.

The reaction is based upon the transformation of aceto-acetic acid into acetone by the action of sulphuric acid.

Salicylates or pyramidon must not be administered prior to the test.

The test may also be made by placing 10 c.c. of antipyrin solution in a 10 c.c. graduated cylinder and adding 20 drops of iron perchloride.

In a similar cylinder place 5 c.c. of urine and 10 drops of iron perchloride and add the N/10 sulphuric acid until the color of the two solutions matches. To calculate the concentration, the amount of sulphuric acid required is multiplied by 20.

In a study of 87 cases of advanced diabetes it was calculated that when the concentration rises above 120 there is a possibility of coma.

Belding, D. L.: The Influence of the Antigen upon the Wassermann Reaction in Pregnant Women. Amer. Jour. Syph., October, 1925, ix, 4.

Report of a clinical and serologic study of the comparative results with plain alcoholic, acetone insoluble, and cholesterolized antigens in the Wassermann test in pregnant women.

Under two methods of fixation comparable results were obtained when the same cholesterolized antigen was used, but considerable variation occurred when different antigens were employed. Approximately one-third as many positives occurred with the plain antigen, and two-thirds as many with the acetone-insoluble antigen as with the cholesterolized antigen.

A strong reaction with plain alcoholic or acetone-insoluble antigens employed in the technics described almost invariably denotes syphilis; a weak reaction is undecisive.

Strong reactions with cholesterolized antigens do not indicate syphilitic specificity but are less likely than weak reactions to be nonsyphilitic. Cholesterolized antigens should be controlled in pregnancy by plain or acetone-insoluble antigens.

The Wassermann tests were all made by both the Massachusetts Board of Health method and the method devised by the author.

Török, G.: Reduction Index of the Urine of Atrophic Infants. Monatschr. f. Kinderh., November, 1925, xxxi, 169.

The observation that the relation between the superficies and body weight is disturbed was the starting point of these investigations, the purpose of which was to endeavor to find some characteristic factor which, in the hands of clinicians, might prove a guide to therapy.

By the reduction index is understood the amount of N/10 potassium permanganate solution reduced by 1 c.c. of urine in 10 minutes' boiling.

The author prefers an indirect method by steam.

In atrophic infants showing a striking gain in weight after receiving carbohydrates, a falling index (lessened amount of organic substances) indicates the start of continuing tissue formation.

Tissue destruction causes a rise in the reduction index.

The author considers the reduction index a delicate gauge of improvement in these cases.

Elwyn, H.: A New Explanation for the Occurrence of Eclampsia in Pregnancy. Amer. Jour. Obst. and Gynec., November, 1925, x, 5.

Elwyn suggests that the clinical phenomena of eclampsia in pregnancy and the histologic changes seen in the liver and kidneys in many ways form a complex which parallels that of acute diffuse glomerulonephritis with acute convulsive uremia, though the two conditions are essentially different.

In pregnancy it has been shown that there is an increased irritability of the neuromuscular mechanism of the uterus. For the function of vasoconstriction there is a somewhat similar anatomic mechanism, the impulses traveling along the same neural pathway as those for uterine contraction.

Elwyn advances, as an explanation of the etiology of eclampsia, a hyperactivity of both these mechanisms in response to various stimuli, thus leading to a general arterial constriction which produces the symptom-complex recognized as eclampsia.

McClure, C. W., Mendenhall, W. L., and Huntsinger, M. E.: Studies in Liver Function, IV. A Procedure for the Uniform Stimulation of the Biliary Flow. Boston Med. and Surg. Jour., December 3, 1925, cxciii, No. 23, p. 1052.

Magnesium sulphate solution, which is mainly depended upon to produce a flow of bile, at times fails to do so, because, when it is improperly used, it causes a depression of the biliary function of the liver.

In a search for a substance which would uniformly stimulate the flow of bile the following procedure was devised:

After the passage of the duodenal tube into the second part of the duodenum, which is verified by the fluoroscope, the subject reclines on the right side, and a mixture of 5 c.c. of oleic acid and 45 c.c. of water is poured through the tube into the duodenum. The proximal end of the tube is closed by a clip for fifteen minutes. Collection of duodenal contents is then begun by siphonage.

The first sample contains oleic acid and frequently gushes out of the tube. It is discarded. Collection for analytical purposes is then begun and continued for thirty minutes. If, at any time during this period, a marked change occurs in the color, a new flask is sub-

stituted and the differently colored contents are collected for a further period of thirty minutes.

Studies were made of 12 normal subjects of both sexes, ranging in age from twenty-three to fifty, and the results of the analyses are discussed in detail.

McClure, C. W., Huntsinger, M. E., and Montague, O. C.: Studies in Liver Function, III. Methods for the Determination of the Furfurol Number and the Bilirubin Concentration of Duodenal Contents. Boston Med. and Surg. Jour., December 3, 1925, cxciii, No. 23, p. 1050.

Determination of the furfurol number.—Reagents: Furfurol (Merck's reagent), sulphuric acid, C. P., Schuchardt's preparation of glycocholic acid.

1. Three-tenths per cent furfurol solution. Dissolve 3 c.c. of furfurol in about 700 c.c. of water in a volumetric flask and dilute to 1 liter.

2. Fifty per cent sulphuric acid. Equal parts of sulphuric acid (Sp. Gr. 1.84) and water. Cool before using.

3. Glycocholic acid standard. Dissolve 100 mg. in about 30 c.c. of N/10 NaOH in a 50 c.c. volumetric flask and dilute with the same solution to 50 c.c.

Method: To 1 c.c. of duodenal contents add 9 c.c. of 95 per cent alcohol and mix. Filter through dry paper. Add 1 c.c. of filtrate to 6 c.c. of 50 per cent sulphuric acid in a test tube. Add 1 c.c. of 0.3 per cent furfurol and 1 c.c. of water and mix by rotation. Place the tube in a water-bath at 67° C. for thirty minutes. Remove and cool under tap water. If necessary, clarify by centrifugation. A slight turbidity does not interfere.

Prepare a standard by adding 1 c.c. of standard glycocholic acid solution to a mixture of 6 c.c. of 50 per cent sulphuric acid, 1 c.c. of 0.3 per cent furfurol, and 1 c.c. of 95 per cent alcohol. This tube is placed in the water-bath and treated like the unknown.

Color comparison is made in a Hellige colorimeter, using the scale reading up from the bottom.

The reading of the unknown multiplied by 2 is arbitrarily termed the furfurol number.

Determination of Bilirubin.—By bilirubin is meant the brownish-colored pigment or mixture of pigments seen in the duodenal contents after stimulation by magnesium sulphate or oleic acid.

Reagents: Ten per cent suspension of calcium hydrate in water. One-half per cent solution of sulphanilic acid (5 grams of sulphanilic acid dissolved in about 800 c.c. of distilled water and diluted to 1 liter in a volumetric flask. Add a few c.c. of chloroform to prevent growth of fungi. The solution is stable.) Two-hundredths per cent solution of sodium nitrite (0.1 gm. in 500 c.c. of water). Two-tenths per cent solution of biliphein (10 gm. in 50 c.c. of chloroform. The solution is stable for at least 2 months). HCl solution: 25 c.c. of C. P. HCl and 75 c.c. of distilled water. Magnesium sulphate solution: 25 grams in 100 c.c. of water.

Method: Place 10 c.c. of duodenal contents, if yellow (5 c.c. if brown) in a 15 c.c. centrifuge tube. Add 1 c.c. of magnesium sulphate solution and 3 c.c. of calcium sulphate suspension and stir with a glass rod. Rinse the rod with water and centrifuge. Decant the clear fluid and add 5 c.c. of water to the precipitate; mix with glass rod, and add 5 c.c. of HCl solution. Mix until all the calcium hydrate is dissolved. Wash the rod with water and centrifuge. The supernatant fluid should be opalescent and should not give any color with the sulphanilic acid-nitrite reagent.

Again decant; add 10 c.c. of alcohol; mix, grinding up the flocculent particles; centrifuge again till fluid is clear. Repeat the alcohol washing three times. After the third washing decant the alcohol; add 3 c.c. of chloroform, and mix with a glass rod. Filter through paper. Add 1 c.c. of this chloroform filtrate to the following mixture, prepared exactly in the order described: 1 c.c. of 0.5 per cent sulphanilic acid solution, 1 c.c. of 0.02 per cent sodium nitrite solution, and 4 c.c. of alcohol. Mix by rotation and allow to stand thirty minutes.

Compare against the color developed in 1 c.c. of standard biliphein solution similarly treated.

The index of bilirubin concentration is expressed in terms of mg. per 100 c.c. of duodenal contents.

Biliphein is a purified bilirubin prepared by Eimer and Amend.

Tandowsky, R. M.: Serologic Studies of Proteinurias: Results of Precipitin Tests Following Injections of Antistreptococcus Serum and of Normal Horse Serum. Jour. Am. Med. Assn., Jan. 23, 1926, lxxxvi, 263.

Proteinuria is neither inevitably significant of nor concomitant with renal disease. It may follow the intravenous administration of large amounts of serum.

The paper reports the results of precipitin tests on the urine of patients receiving antistreptococcus serum and from dogs after the intravenous administration of normal horse serum.

Rabbits were immunized against the serum and their serum used when the titer was not less than 1:60,000.

To 3 c.c. of urine 0.2 c.c. of rabbit serum was added and readings made after one hour at room temperature.

By this procedure the elimination of horse serum was detected in the urine within thirty minutes. The greatest concentration was found within the first eighty minutes.

The proteinuria continued for approximately four hours.

Prucha, M. J., and Brannon, J. M.: Viability of B. Typhosum in Ice Cream. Jour. Bacteriol., January, 1926, xi, No. 1, p. 45.

Reports of a bacterial count of ice cream containing B. typhosum artificially introduced.

After five days a reduction of 90 per cent in the number of typhoid organisms was noted; after twenty days the reduction was more than 95 per cent. After one year one germ in a thousand survived; after two years one germ in ten thousand.

Viable typhoid bacilli were found after two years and four months, when the examinations were terminated because the sample had been exhausted.

Storage was at an average temperature of minus 4° F., and the experiment demonstrates that the typhoid organism is viable after prolonged exposure to this temperature.

Roffo, A. H.: Serologic Test for Cancer. Prensa med. Argentina, October 10, 1925, xii, 481.

To 2 c.c. of clear serum add 5 drops of 0.5 per cent solution of neutral red. Normal serum remains yellowish; cancerous serum rapidly turns red.

The reaction is based upon the relation assumed to exist between the colloidal content of the serum and the occurrence or disappearance of fluorescence.

The test was devised in conjunction with an experimental study of tumors in rats.

Crowell, M. J.: Morphological and Physiological Variations in the Descendants of a Single Diphtheria Bacillus. Jour. Bacteriol., January, 1926, xi, No. 1, p. 65.

From the study of single cell cultures of B. diphtheriae the following conclusions are formulated:

1. Morphologic types of the diphtheria bacillus have no hereditary significance and no relation to virulence.
2. The descendents of toxic organisms may be toxic or nontoxic.
3. The nontoxic strains are direct mutations from toxic strains.
4. The nontoxic strains are permanently nontoxic.

BOOK REVIEWS

(Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.)

*Applied Biochemistry**

BIOCHEMISTRY has rapidly advanced from a pure to an applied science. In its earlier stages the authoritative works, such as Hammerstein's were written along the line of abstract chemical studies. Perhaps the first outstanding attempt to correlate the purely scientific with the clinical was that made by Mathews. The next great step in this correlation was typified in MacLeod's "Physiology and Biochemistry in Modern Medicine."

The work of Morse carries the development a step farther. He emphasizes perhaps more pure biochemistry and less the clinical aspects than have some other writers. Illustrations and illustrative diagrams are numerous and remarkably well done. A distinct innovation is the inclusion of photographs of leading biochemists which are scattered through the book in the sections in which they have done outstanding work. In this way the student can come to think of the men of whose work he reads in a less abstract way. Probably it would be impossible to accord this honor to all who deserve it, but we regret not seeing a photograph of Dr. Folin, whose name is perhaps better known throughout the profession at large than any of the others.

In his introduction the author has an interesting "family tree," tracing the origin of American biochemistry through some of the lines of its development from Lavoisier in France and Liebig and Voit in Germany, through their pupils such as Atwater, Lusk, Folin, Mandel, Chittenden, Jones and Mathews, and on down through the students of these latter, who have contributed outstanding advances to our knowledge of biochemistry. While made up as an interesting genealogic table it constitutes one of our best briefs for the good effect of *environment*, expressed in this case as a thorough "grounding" under the masters.

The body of the book is arranged in the customary subdivisions: enzymes and their action, the body and its maintenance, glucids, lipids, protids, the special chemistry of the tissues, chemistry of common foods, digestion and absorption, intermediate metabolism, nutrition, the energetics of nutrition, metabolic adjuvants, the excretions, methods for the determination of blood constituents, and metabolic studies on blood and urine.

Scattered through the work are found experimental exercises for the student, illustrative of points brought out in the text. In the appendix the author has incorporated a comprehensive list of reagents and various tables, such as the hydrogen-ion concentration of various substances, characteristic biochemical configurations, scheme for the analysis of milk, table of magnifications, tables for metabolic determinations and vitamin tables.

*Applied Biochemistry. By Withrow Morse, Ph.D., Professor of Physiological Chemistry and Toxicology, Jefferson Medical College, Philadelphia. Cloth. Octavo. 257 illustrations. Pp. 958. Price $7.00 net. W. B. Saunders Company, Philadelphia and London. 1925.

*Handbook of Pathology**

THIS book was written, in the words of the author, "with the object of presenting in a short, concise form the more important and salient points in pathology," and it is intended primarily for the medical student.

The task outlined has been very successfully accomplished.

The arrangement of the book is, as far as possible, along etiologic lines and, where this is impossible, on the basis of the character of tissue change whereby lesions of similar or allied nature are grouped together.

Beginning with the morphology of cells, degeneration and allied changes (edema, hyperemia, atrophy, hypertrophy, hemorrhage, pigmentation), the reaction to inflammation, etc., are next considered.

Diseases due to bacteria, filterable viruses, metabolic disturbances and those affecting the blood are next discussed, the final chapter of 194 pages being devoted to a consideration of tumors.

Only the more common conditions are described, special attention being given to the morbid anatomy.

The general plan includes a description of the etiology and gross and microscopic features together with such particular features as are of importance in the individual case.

The volume is very well and clearly written. The illustrations, mainly microphotographs, are well chosen and well reproduced.

While not supplanting the more complete and standard texts on this subject, this book furnishes a very satisfactory and useful concise review and reference work of use both to the student and to the physician. It can well be recommended for this purpose.

Parasitology for Medical Students†

PARASITOLOGY is a subject the mastery of which requires more extensive and profound study than it receives from the average practitioner. It is essential, nevertheless, that some knowledge be had of the salient features of those parasites which are of practical importance from the standpoint of the physician, and in this small volume the author has endeavored, with marked success, to present a brief but practical account of such parasites, their life history, salient characteristics, and a brief discussion of the diagnosis, prophylaxis, and treatment of the diseases caused by them.

The resultant compact volume is very practical.

The descriptions are clear and well written. The illustrations, all micrographs taken by the author, are well reproduced and well chosen.

No one will become a parasitologist through the perusal of this book, but every practicing physician can read it with profit.

*Handbook of Pathology. By C. Y. Wang, Professor of Pathology, University of Hong Kong. Cloth. Octavo. 282 illustrations, 6 in colors. 513 pages. Price $7.00. William Wood and Co., New York.

†Parasitology for Medical Students. By A. M. Kennedy, Professor of Medicine, University of Wales. Cloth. 61 microphotographs. 1 colored plate. 138 pages. Price. $3.00. Oxford University Press.

The subjects covered are: Insects and Arachnoids, Tapeworms or Cestodes. Flukes, or Trematodes, Roundworms or Nematodes, Protozoa, and Spirochetae and Fungi.

Under each heading only those parasites of predominant importance as etiologic agents of diseases relatively common in man are considered and the form followed is the same for all: description, diagnosis, prophylaxis and treatment.

The treatment is briefly given, generally only one method being described. For the treatment of syphilis the reader is wisely referred to larger works.

This is a book which can be added to the library of any practitioner with little doubt of its applicability to his work. For the medical student, for whom it is especially written, it is one of the best small manuals which the reviewer has seen.

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*Insects and Diseases of Man**

IN this book the author presents in a concise and practical way a discussion of medical entomology for the student, the physician, and the health officer.

No attempt is made to cover the subject in extenso. The aim, rather has been to consider the subject from the standpoint of the essentials for public health work. The task attempted has been very satisfactorily achieved.

The book is divided into two parts: Part I (227 pages) is devoted to the classification, identification, anatomy, life history, etc., of insects of importance in medical entomology, and it includes a section on Arachnidae and one on rodents, together with a short chapter on technic.

There are numerous clear-cut line drawings, many of which were made by the artist of the hygienic laboratory, under the author's personal supervision.

Part II (98 pages) discusses the diseases in the human being which are transmitted by arthropods. It contains a practical discussion of the subject, especially with regard to prevention and control, treatment of carriers, etc., and instructions concerning the collection of material and the details of the necessary field investigations. The book is eminently practical and should be of great use to the student, the health officer, and others interested in or engaged in this work.

Micromethods†

THIS is a collection of the methods used by Pincusson in the city hospital in Berlin. Some of them are his own and some are his modification of other methods. Most of the determinations are made by means of the colorimeter or the nephelometer, and most of the organic and the inorganic constituents of the urine and blood are covered. Hijmanns van den Bergh's test for bile pigments and the indicator method for hydrogen-ion concentration are

*Insects and Diseases of Man. By Carroll Fox, Associate Professor Hygiene, Medical School, University of the Philippines. Cloth. 92 illustrations. 219 pages. Price $4.00. P. Blakiston's Son and Co., Philadelphia.

†Micromethods; quantitative determination of the constituents of urine and of blood in small quantities. By Ludwig Pincusson. Geo Thieme, Leipzig, 1925.

included. Very few of the tests are credited to American authors, though Folin's method for amino nitrogen and for blood sugar, Folin and Wu's method for uric acid, Benedict's method for uric acid, and van Slyke's method for alkali reserve of the blood are found. The author describes and gives a picture of a modified torsion balance by which a weighing accurate to one milligram can be made in a few seconds. This is especially useful in some of Bang's methods in which a piece of moistened filter paper must be weighed quickly before evaporation takes place.

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*A Textbook of Medical Diagnosis**

THIS book, intended as a practical aid to the practitioner in the study of the diagnostic problems with which he is daily confronted, is an extensively revised third edition of a work originally published in 1911 by two well-known teachers, and it embodies the practical application of their combined experience.

Recognizing that the diagnosis of disease is evolved from the recognition and evaluation of distinctive symptoms for the detection of which many varied and specialized forms of examination may be utilized, the authors propound very clearly a systematic plan of investigation for the clinical study of disease, in which the salient features of each condition are first separately considered and then are grouped under subheadings: "Laboratory Diagnosis," "Summary of Diagnosis," and "Differential Diagnosis," the latter frequently in the form of or accompanied by succinct and useful tables.

The text amply reflects the professional experience of the authors.

The subjects covered include: Respiratory diseases (142 pages); circulatory diseases (257 pages); diseases of the digestive system (246 pages); the urinary system (82 pages); acute infections (239 pages); animal parasites (47 pages); constitutional diseases (20 pages); endocrine diseases (104 pages); and nervous diseases (200 pages).

The discussions are clear, well written, and eminently practical. The section concerned with the clinical utilization and application of blood chemistry might, perhaps, be expanded with benefit.

The volume is concerned entirely with clinical methods of examination; laboratory methods are referred to, and their clinical applicability to the problem at hand and their interpretation are discussed, but the technic is wisely left to volumes particularly concerned with these methods. The Wassermann technic, nevertheless, is described in full, and, as this is a matter beyond the province of the practitioner, it seems superfluous. Precipitation reactions in the serodiagnosis of syphilis are both wisely and conservatively discussed and will be in entire accord with the statements made by the majority of serologists. The section on constitutional diseases is somewhat compact.

The book is easy to read and well printed. The illustrations are numerous, well chosen, illustrative, and well reproduced.

*A Textbook of Medical Diagnosis. By J. M. Anders and L. N. Boston. Ed. 3. 555 illustrations, 21 in colors. Pp. 1366. Price $12.00 W. B. Saunders & Co., Philadelphia.

There are almost no typographical errors, though one notes *precipitan* for precipitin on page 383 and *Hellar* for Heller on page 521.

The volume should be a useful addition to the reference library of the practitioner, to whom it is addressed.

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*Handbook of Biological Methods**

ABDERHALDEN'S handbook is one of those immense compilations which can be published only in Germany. Every library should have it, and every one doing biologic work of any kind should have access to it, as it contains everything he may want to know about methods of work.

Lieferung 142 contains 260 pages, is written by Francis G. Benedict, the well-known investigator of metabolism, and contains a description of the respiration calorimeter in the nutrition laboratory of the Carnegie Institution in Boston. Full and detailed descriptions of the methods of determining the amount of oxygen consumed, the carbon dioxide excreted, and the heat given off are given, together with mathematical formulas and tables to simplify the necessary calculations. The fact that it is written by Benedict is sufficient assurance of its high quality and accuracy.

Lieferung 158 contains special methods of colorimetry ranging from a colorimeter for work with large animals, by J. W. Capstick of Cambridge, England, to a microcalorimeter for determining the heat produced by cells, such as red blood corpuscles, eggs of the sea urchin, bacteria, yeast, etc., by Otto Meyerhof of Berlin. There is also an article on the method of determining the gas exchange in large animals, by W. Klein and Marie Steuber of Berlin, and a description of an electrical compensating calorimeter by Paul Hari of Budapest.

Lieferung 143 contains an article by R. Grammel of Stuttgart on the theoretic basis of the mechanics of joints. It is a mathematical treatment of the mechanics and the dynamics of the movements of joints and of the masses moved by them. There is also a short article by E. Hirt of Münich on a graphic method for demonstrating the cause of normal and pathologic voluntary movements, from a simple finger movement to the complicated and coordinated movements of writing.

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*Handbook of Biological Methods. By Emil Abderhalden Division IV, Part 10, Part 3, (Lieferung 142); Part 4, (Lieferung 158); and Division IV, Part 10, Part 3, (Lieferung 142), Urban and Schwarzenberg, Berlin, 1925.

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EDITORIALS

Laennec
1781-1826

LAENNEC, like Osler, was of Celtic origin. A great deal in common can be noted in the lives of these two men. Both, as boys, delighted in nature studies and in learning. The Gaelic and the humanities fascinated them. As physicians, both men delighted in pathologic anatomy in the search for Truth and to each the study of tuberculosis afforded absorbing interest. Both of these famous physicians made exact observations and were averse to speculation. To each, medical history was of the greatest importance, and with it they illuminated their excellent lectures and treatises. While Laennec immortalized himself by his anatomic studies and clinical writings and the invention of the stethoscope, it was Osler who first suggested the binaural instrument. Laennec resembled another Celtic physician, Oliver Goldsmith. Both of poetical temperament, they had the same passion for natural history and for playing the flute.

Laennec, as a boy, was fortunately brought up by an uncle who was a physician in Nantes. It was this uncle who, regretting his own scant knowledge of Greek, encouraged the boy to master this language, so that later in life he could read Hippocrates in the original.

Just as the father and mother of Pasteur had worried over their son's passion for pastel drawing, so we find the uncle of Laennec anxious over the boy's delight in, and time spent upon, natural history and poetry. It was no doubt the attention to detail which Laennec thus cultivated that enabled him to discover the subdeltoid bursa, the fibrous capsule of the liver, peritonitis, melanotic cancer, bronchiectasis, the unity of tuberculosis, and many other conditions which he was the first to describe.

Laennec himself suffered many diseases, and one of these may have been pulmonary tuberculosis. His life was a continued struggle against asthma. He employed bizarre remedies, such as sitting at his studies with his body naked. We recall that Benjamin Franklin, who had also suffered from a pulmonary complaint, had advocated and practiced a similar measure. Laennec wrote an accurate description of an attack of angina pectoris from which he suffered. Gout, dizziness, and neurasthenia, he also described, as a victim, and to remedy these "he wearied his legs to rest his head."

Laennec was not so austere as he wished to appear. Called upon for an impromptu toast at a dinner at Soissons, he stated that he was only acquainted with songs pertaining to drinking. He "toasted" tobacco and the inventor of the bottle, quoting Hippocrates that water was the cause of all evil!

Laennec was fortunate in his early medical-student days, in Paris, to come under Corvisart, who was then teaching clinical observation and the method of percussion of Auenbrugger. The famous physiologist, Bichat, was another teacher; and Bayle attracted Laennec by his love of natural history and of religion, and through his careful anatomic study of the tubercle. It was Laennec's faithful devotion to autopsy study that enabled him to become the foremost physician of his day. Pasteur has taught us to reverence great men, and it is in this spirit that the centenary of Laennec's death is now being observed by many medical societies. Laennec, unlike Pasteur, did not live to reap the reward of his genius.

The immortal work on auscultation, which was published in 1819, was met with ridicule by Broussais, and physicians who employed the stethoscope were called charlatans. We, however, as physicians, can well attest the victory of Laennec, when we recall a paragraph from the introduction to his treatise: "I shall consider it ample, yea, more than sufficient reward for my labor, if it should prove the means by which a single human being is snatched from untimely death."

—*G. B. W.*

Laboratory Diagnosis

PROGRESS in medical science is more often the end-result of perspiration than of inspiration, of evolution than of revelation.

The practice of medicine revolves around and evolves from the study of disease and its manifestations; it begins with diagnosis and progresses with

an understanding of the underlying pathology and mechanism of symptom production.

To this detailed study of disease have been applied all the resources of a varied array of sciences, and through their application has arisen a variety of procedures, all of which, to be of use, must be interpreted and clinically evaluated in terms of the individual problem.

Chemical, bacteriologic, and serologic studies have been of inestimable value in the elucidation of many heretofore obscure factors of etiologic and pathologic importance, and from their careful analysis certain general dicta of practical value in diagnosis have been formulated. There is an unfortunate tendency, however, which cannot be too often commented upon nor too strongly decried, to regard the test as the paramount thing, to overlook the vital necessity for its interpretation.

There are too many and too glib references in current medical conversation and current medical literature to "laboratory diagnosis" and "diagnostic tests."

On the one hand, there are those who tend to rely almost entirely upon the results of laboratory investigations, and, on the other, those who remind us that laboratory aids often do more than furnish a verification of that which the well trained bedside clinician can discover through the peculiar technic or devices characteristically his own.

Neither is entirely right, and it is unfortunate that the bulk of the profession is to be found ranged either on one side or the other rather than in the middle of the road.

There are no pathognomonic laboratory tests! The purpose of the laboratory is not to make diagnostic ventures!

No one is more insistent than the laboratory worker in proclaiming from the housetops the essential necessity for a correlation of laboratory and clinical data and their joint interpretation. The laboratory investigations are only *one* phase in the study of the individual case, one means of securing information frequently obtainable in no other way, one more item among the many whose sum total is the diagnosis.

It is by the well trained bedside clinician that the results of laboratory studies are most often intelligently interpreted and laboratory studies most skillfully applied, but even the well trained bedside clinician sometimes fails to detect hidden foci of spirochetae whose presence is revealed by the complement-fixation reaction; may fail to differentiate the early chancre from the chancroid in the absence of the dark-field illuminator; may overlook the presence of immune bodies detected by the Schick or Dick tests; may experience some difficulty in the immediate differentiation of uremic from other comatose states without the assistance afforded by blood chemical determinations, and so on.

It is when these and other similar situations arise that the peculiar technic or devices characteristically the clinician's may be inadequate to diagnosticate the condition clearly and completely, and then the laboratory is of the greatest value and assistance.

Unfortunately, there exists a modicum of those not so skillful or so well trained, and it is by these that a blind reliance is too frequently placed upon the laboratory and its tests.

The laboratory requires no clinical accolade. Properly used and skillfully applied to the scientific study of clinical problems, it furnishes both valuable and useful information and is an indispensable part of the study of those problems.

When improperly applied and its results abused or unwisely interpreted, the onus rests upon the clinician and not upon the laboratory.

—*R. A. K.*

CORRESPONDENCE

To the Editor

Dear Sir:

Every clinical pathologist certainly must agree with Dr. Haden's editorial statement in the April number of your Journal: "That there are wide differences in normal hemoglobin values as given by authors and workers." This has proved so difficult in correlating hemoglobin estimations on the same patient, when made in different places, that in this hospital over two years ago, we began reporting all hemoglobin estimations in grams per 100 c.c. For those who are wedded to a given standard, it is easy to translate the approximate percentage by a simple multiplication, but many soon acquire the ability to visualize the hemoglobin value in grams, rather than percentage, just as the cell counts, which are given in actual values, have long since acquired definite concepts in the physician's mind.

I would suggest, therefore, that instead of trying to arrange an authoritative hemoglobin percentage standard, the American Association of Clinical Pathologists and your Journal can give even more valuable service by emphasizing the need of reporting hemoglobin in grams per 100 c.c.

Sincerely yours,

(Signed) Edward B. Krumbhaar,

Director of Laboratories, Philadelphia General Hospital.

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The following officers for the year 1926 were elected at the Fifth Annual Meeting:

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The Society maintains a Service Bureau for its members. Any clinical pathologist wishing to make a change should communicate with the Secretary.

Kindly report any change of address to the Secretary.

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CLINICAL AND EXPERIMENTAL

A STUDY OF THE ORGANISMS OCCURRING IN TWO CASES OF EROSIO INTERDIGITALIS*

By G. V. Stryker, M.D., and Moyer S. Fleisher, M.D., St. Louis, Mo.

Erosio interdigitalis is considered to be a rare disease, but probably occurs more frequently in America than the literature indicates. The clinical characteristics and the ease with which the organism may be found in the scale make the diagnosis comparatively easy.

The two cases from which the material for our studies was secured, occurred in a series of 450 cases at the Skin Clinic of the St. Louis City Dispensary.

Both patients were elderly Jewish women of Russian nativity. Each did her own housework, including the washing of dishes and clothes. Soap was used by both patients in the clothes washing and general cleaning but not in the washing of dishes.

REPORT OF CASES

Case 1.—*History.*—On July 22, 1924, Mrs. F. E., aged sixty-four years, presented herself at the clinic complaining of itching lesions between the fingers. The lesions had been present for ten months, and were located on the web of the third interspace of the right hand, and the third and fourth interspaces of the left hand. All lesions had developed at the same time, progressed to a certain point, and had then remained stationary.

Subjective symptoms.—Intense itching at times.

Objective symptoms.—The lesions were sharply defined, superficial and confined to the web of fingers. They had never extended to the palm or dorsum of the hand. They were covered with a loose layer of white, sodden epidermis, which could be removed easily, without discomfort to the patient. When this was done, there was disclosed a smooth, shiny, red

*From the Department of Bacteriology and Hygiene and the Department of Dermatology, St. Louis University School of Medicine.

Received for publication, September 26, 1925.

epidermis, without fissures, vesicles or evidence of exudation. The patient's general health was good. No other dermatologic lesions were present. (Figs. 1 and 2.)

CASE 2.—*History.*—Mrs. I. S., aged sixty years. Born in Russia, but a resident of America for eighteen years. This patient was first seen at the Skin Clinic of the St. Louis City Dispensary on August 7, 1924. She presented a lesion on the web of the third interdigital space of both hands. The one on the right hand had been present for four years; the one on the left for a year and a half. As in the first case, the lesions had neither extended nor retrogressed since the onset, and had remained confined to the webs of the fingers.

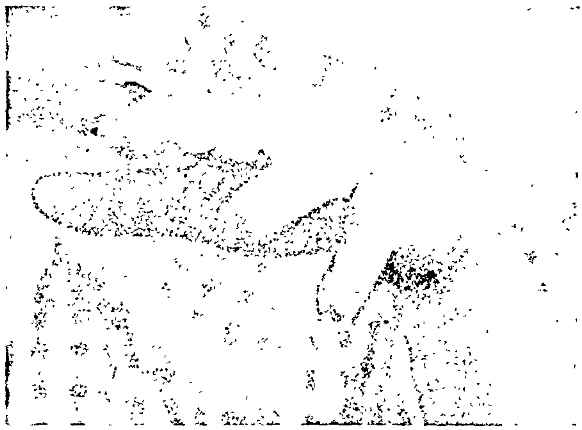

Fig. 1.—Shows the lesion on the left hand of patient "F.E."

Fig. 2.—Shows the lesions on the right hand of patient "F.E."

Subjective symptoms.—Occasional slight itching.

Objective symptoms.—At the time of the first examination the lesions compared very closely with those described by Mitchell.[1] They were dry, shiny, red, and surrounded by a "collarette of scales," which were removed with difficulty and the removing caused considerable pain to the patient. When, however, the patient returned one week later, on the day following her wash day, the red surface was covered with a white, sodden epidermis which could be removed without pain. There were no other dermatologic lesions present. (Fig. 3.)

Microscopic examination.—The scales of both cases when treated with potassium hydroxide solution revealed many round and oval double contoured organisms.

The literature pertaining to studies of the organisms isolated from so-called cases of Blastomycosis interdigitale or Intertrigo saccharomycetica, or Erosio interdigitalis blastomycetica seu saccharomycetica, does not furnish very satisfactory evidence concerning the organisms actually dealt with by the various authors. In general, most of the authors refer to the organisms found in these types of skin lesions (whether they occur as interdigital lesions or elsewhere on the skin of other parts of the body) as yeasts, yeast-like organisms or blastomyces. Possibly a review of the earlier studies of the yeast-like organisms causing lesions of the skin or occurring in the skin will be of interest.

It has been noted by a number of observers that in cases of thrush, organisms similar to or identical with those causing the lesions in the mouths of the infants can at times be found in skin lesions and in the feces of such cases. Thus in 1907, Moro[2] noted the occurrence of so-called "soorpilze" in

Fig. 3.—Shows the lesions on both hands of patient "I.S."

skin lesions about the anus of children suffering with thrush and he further found the "endomyces" in the feces of such cases. Chiray and Sartory[3] found similar organisms in the stools of poorly nourished infants. Ibrahim[4] found similar organisms about the anus, and in lesions of the skin, both intertriginous (between the fingers or toes) and nonintertriginous—which organisms he did not, however, definitely consider to be the causative agents of the disease.

Whitfield,[5] who reported a case of intertrigo in 1908, simply called the organism demonstrated a yeast, and apparently made no further effort to identify the organisms.

Gougerot and Goncia,[6] who reported a case of intertrigo in 1914, describe the organism as being like that found in thrush but not the Endomyces albicans. They describe the culture of the organism on solid media as creamy and raised; they studied the cultural qualities on various media, and also made a study of the agglutinative and complement-fixation qualities of the organism as compared to Endomyces albicans and Sporotrichum beurmanni

and Sporotrichum gougeroti. They describe their organism as spherical or ovoid and showing budding. They call it a yeast-like organism but go no further in their identification. Since they saw no asci or ascospores, their organism cannot be classed as a saccharomyces.

Hudelo and Montlaur,[7] and later Hudelo, Sartory and Montlaur[8] reported a number of cases in which they isolated from the skin lesions (usually intertriginous) yeast-like organisms, which, in the later article, are classed as saccharomyces. In the scales they demonstrated oval cells arranged in filaments. The spherical to ovoid organisms showed budding at one and occasionally at both poles, and they were able to demonstrate asci and four ascospores in the sac. The cultures showed pseudomycelia at times but no chlamydospores. The growth was creamy, and in color white to "café au lait." The organism fermented maltose but had no action on lactose, galactose or levulose, and did not liquefy gelatin.

They also found a similar organism, identical in cultural and biochemic qualities, but showing no asci or ascospores, with more abundant pseudomycelia and much like Endomyces albicans. Whether these latter were found associated with the saccharomyces in the lesions, or whether the two types were found in lesions from different cases is not clear from their report.

Fabry[9] and his coworkers state that yeasts were found in cases of Erosio interdigitalis blastomycetica. The description of the morphology and cultural qualities of the organisms by Berendsen[10] is quite inadequate to permit of assigning these organisms to any specific genus or even suggesting their biologic classification.

Tanner and Feuer[11] described and studied quite thoroughly an organism isolated from an infection of the skin. The organism was round to ovoid, 3.5 to 6.5 μ and showed no spore forms. It grew on glucose agar as a white, slimy, luxuriant growth; coagulated and digested casein but did not render it acid, and fermented glucose completely, but did not act upon sucrose. They classified the organism as an Endomyces albicans.

Engman[12] in 1920 described an infection of the skin due to an organism which he classed as a hyphomycetes or monilias—as either a monilia or oidium and belonging to the genus Botrytis. Unfortunately, he does not give a description of the cultural characteristics of the organism, but evidently depends upon authoritative advice for the identification.

Castellani and Chalmers,[13] in their *Manual of Tropical Medicine,* mention as potential causes (agents) in Intertrigo saccharomycetia both saccharomyces and also fungi of the genus monilia.

Stechel[14] in 1921 speaks of the organisms found in interdigital erosions as yeasts, but gives no cultural description nor definite basis for this nomenclature.

Weidman[15] calls attention to the morphologic characters noted in the organisms in skin scrapings from lesions between the fingers, toes and about the anus, and states that these grew as yeasts but showed mycelia in old glucose agar plates. One of the photographs of the organisms shows an appearance practically identical with the typical monilia (or soorpilze) growth

form. He writes of the resemblance of yeasts to hyphomycetes, so presumably he classes these organisms as yeasts.

Mitchell,[16] writing on Erosio interdigitalis blastomycetica, does not consider specifically the organisms causing this type of lesion.

Greenbaum and Klauder[17] separate the yeasts causing intertriginous infections of the skin into four types, and we give below their tabulation of the points of differentiation as they have stated them:

	TYPE I	TYPE II	TYPE III	TYPE IV
Glucose Agar				
Surface	Wrinkled	Smooth	Smooth	Smooth
Color	Cream	White	Yellow	Salmon
Raulin Media	Sediment	Sediment	Sediment	Sediment
Glucose	0	0	0	+
Levulose	0	0	0	0
Maltose	0	0	0	0
Mannite	0	0	0	0
Litmus	Acid	Acid	Acid	*Amphoteric*

They classify them all excepting the second type as saccharomyces and this exception they classify as a cryptococcus. Ascospores or asc sacs were demonstrated in Types I, III and IV. In studying yeasts from normal skin they found these same types present in a number of individuals; thus in 35 of 150 individuals examined, one or another of these four types of yeasts were demonstrated in culture. In a series of seven interdigital lesions these *same types of organisms were present; thus Type I and Type II each in two* cases, and Type III in three cases.

It would appear then from this review of the literature that: First, a large part of the work concerning so-called yeasts as agents in causing diseases of the skin is inadequate to permit of drawing any conclusion as to the actual type of organisms causing the disease. Second, that apparently true saccharomyces (producing asci and ascospores) may be the causative agents in intertriginous conditions, as shown by Hudelo, Sartory and Montlaur, and by Greenbaum and Klauder. Third, that in infants affected with thrush apparently the same organisms (endomyces or monilia or oidia) which cause the lesions of the mouth may also cause intertriginous lesions. Fourth, that *apparently monilia or similar* organisms may cause lesions of the skin (intertriginous or otherwise), even when there is no thrush, as shown in the reports of Castellani and Chalmers, Tanner and Feuer, and Engman.

MYCOLOGIC STUDIES

We have isolated and studied organisms (fungi) from the skin scrapings (scales) of both of the above cases, and have also isolated either similar or identical organisms from the stools in both cases. Thus in the case I. S., the two organisms (No. 203 skin and No. 203 stool) are *apparently identical*, while in the case F. E. there seem to be slight differences between the organism isolated from the skin (No. 201 skin) and that from the stool (No. 201 stool). *The two organisms isolated* from the skin are very similar, although probably not identical. Thus the skin organisms from I. S. and F. E. and the stool

organism from I. S. are all very similar, while the stool organism from F. E. differs somewhat, and therefore must be described separately.

The three organisms, No. 201 skin, No. 203 skin, No. 203 stool, are spherical to ovoid, measuring about 4 to 5 μ by 2.5 to 4 μ. When stained with methylene-blue or thionin, the larger forms are unstained or show only a faint staining which is most marked at the periphery and which suggests a granular structure of the cell; otherwise the cells, stained in this manner, show little or no evidence of structures such as a nucleus or vacuoles. The smaller forms show a fairly deep staining at the periphery which tends to be more marked at the poles, or which may be confined to limited parts of the periphery, or

Fig. 4.—Culture No. 203 st. Stained smear from Sabouraud's media, four days' growth, showing spherical, irregularly stained cells and also a few unstained cells. Mycelium also seen. Note the similarity between the forms seen in No. 203 st. and No. 201 st. after four weeks' growth.

the staining may appear darker in the peripheral part and faint in the remainder of the cell. The variation in staining is very marked so that we may run the gamut from cells which are practically unstained to others which are quite diffusely stained in a rather uneven, granular fashion. On the whole the larger forms stain less deeply than the smaller forms. In old cultures there are apparently more of the larger, paler forms than in the young (twenty-four-hour) cultures. (Figs. 4 and 5.)

We find also a few longer, thinner cells, varying from no longer than the larger yeast-like cells to two or three times their length, and in width

usually about half the size of the yeast-like cells. These longer cells which are apparently mycelial cells stain irregularly and usually rather faintly, and show one or more refractile bodies within the cells. Usually these mycelial forms are seen in cultures from four to eight days old (Fig. 6).

In the unstained specimens we note forms essentially similar to those seen in the stained specimens. We find large cells with a small amount of granular matter at the periphery; these cells are spherical rather than round. The smaller forms are ovoid, very variable in size, show a double contoured periphery and contain one, or occasionally two bright spots (large when only one, and small when two are present). These bright spots—probably

Fig. 5.—Culture [illegible] ...ouraud's media. Four weeks' growth, showing densely packe[illegible] cells which usually do not stain. Note the extreme variation in the [illegible] cells, some suggesting the so-called durable forms.

nuclei—are at times centrally located, at times lie toward one pole of the cell; they are usually round and are probably from 0.5 to 1.5 μ in diameter. Spores have not been demonstrated in any of the cultures. In each cell there may be noted a dark, actively motile granule which is present in every cell, with the possible exception of some of the very largest forms.

When the smears are fixed with Bouin's solution and stained with hematoxylin (Harris') we note a very faintly stained large, round or spherical area toward the center of the cell; occupying, in some cells, the greater part of the cell, in others, only a limited portion. These masses are usually clear in the center, or may show a few granules stained with the hematoxylin, but

show a definite peripheral staining; there is little question but that they are nuclei. In many cells several nuclei may be seen and practically always in the elongated mycelial forms of cells we find two or more nuclei. We see also, usually toward one pole of the cell, a mass or several masses, usually granular and more or less deeply stained. These lie in close contact with the nucleus and they vary very markedly in size and outline. They seem to correspond possibly with the vacuoles.

When the yeast cells are examined in hanging drop with a weak solution of neutral red, the structure which we have called the nucleus is un-

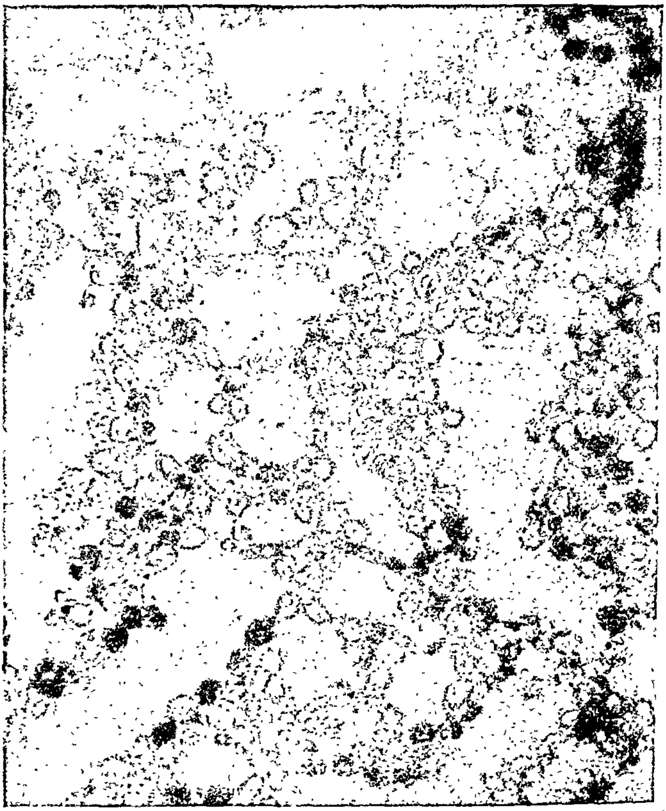

Fig. 6.—Culture No. 201 sk. Stained smear from Sabouraud's media; four days' growth. Showing the typical spherical, stained or unstained cells and also a few mycelial articles.

stained, visible as a highly refractile body, but at one pole of the cell, in contact with the nucleus or partly covering it, we find one or two faintly staining, pale red masses, varying very considerably in size and outline; sometimes as large as the nucleus or again very much smaller; sometimes round or spherical, again irregular or somewhat triangular. It appears probable that these represent the vacuoles of the cells.

We found also in a number of the specimens, in either hanging drops or smears, a varying number, usually few, of very large, spherical cells which take none of the stains and which probably are the so-called "Dauerformen" or durable cells.

In these organisms (No. 201 sk., No. 203 sk. and No. 203 st.) then we find usually spherical forms, a few elliptical forms and occasionally mycelial forms. The cells show a double contoured cell wall, and apparently contain a nucleus or several nuclei, one or more vacuoles, one or two dark motile granules, and possibly a varying amount of granular material. We have never been able to demonstrate spores in any of these organisms, nor have we seen spore sacs. About all that we can state from this study of the morphology of the organisms is that they are not to be considered as ascomycetes.

The cells of the other organism (No. 201 st.) differ from those of the three just described in that the majority are oval or elliptical rather than

Fig 7.—Culture No. 201 st. Stained smear from Sabouraud's media, four days' growth, showing the oval, deeply stained cells.

spherical, this difference being particularly marked in the younger cultures. If, however, we examine old cultures (four to eight months old) we find that most of the cells have the same form and appearance as those of the three strains first described.

The cells of No. 201 st. also stain more diffusely and more deeply with all stains (methylene-blue, thionin, fuchsin and hematoxylin) than do the others; the staining, however, is not even but is rather granular. There is considerable variation in the depth of the staining of individual cells. In some cells we see an unstained, rather refractile structure, usually spherical, which is in all likelihood the nucleus. Any other morphologic features are

masked by the staining of the granular matter within the cells. We find also in smears from these organisms mycelial forms such as were noted in the other organisms. Again no ascospores have been seen (Figs. 7 and 8).

Examined in the hanging drop, whether suspended in broth or in neutral red solution, the appearances of the structures within the cells of No. 201 st. are the same as in the other three organisms. The only thing then that differentiates culture No. 201 st. from No. 201 sk., No. 203 sk. and No. 203 st. is the cell outline and the reaction to stains in the earlier periods of growth. A small number of oval, granular staining forms, however, are seen in the young cultures of the three latter organisms, similar to the forms

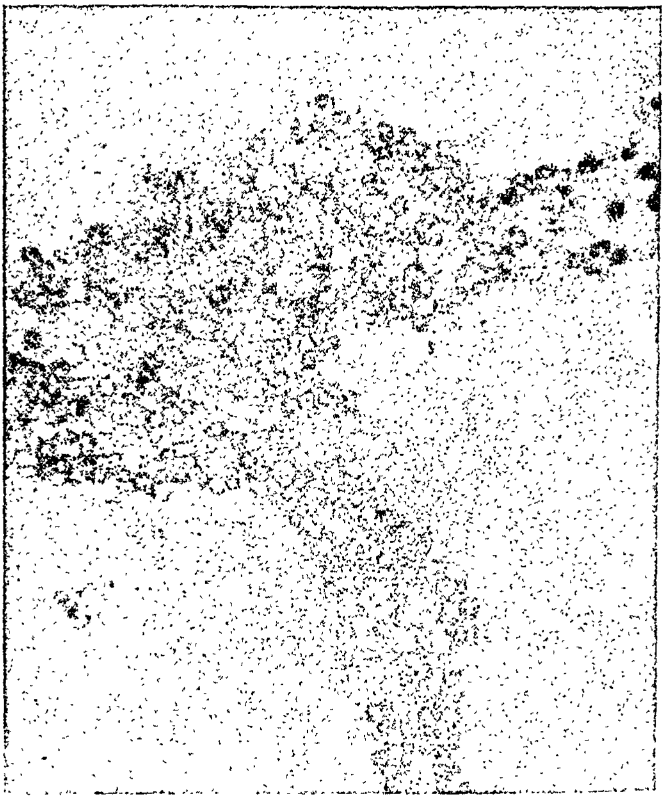

Fig. 8.—Culture No. 201 st. Stained smear from Sabouraud's media, four weeks' growth, showing spherical forms, some unstained, some irregularly stained.

seen in No. 201 st., and furthermore in old cultures of No. 201 st., many of the cells assume the spherical forms of the other three; it is therefore difficult to separate these four organisms on a morphologic basis and it is possible that different rates of growth or a tendency to adhere to a particular form, representing possibly a stage of the life cycle of the organism, may account for the morphologic differences noted above.

The cultures of the four organisms on Sabouraud's media (using Difco peptone and Difco Standardized Maltose) are all very much alike, excepting that again the strain isolated from the stool of patient F. E. differed at times from the other three. The usual growth was profuse, white, and raised,

the edges were rather sharp, and the cultures showed only a slight tendency to spread over the surface of the media. The surface of the growth shows slight irregularities but no distinct wrinkling; it was glistening and appeared moist. No development suggesting aerial hyphae was noted. At times outgrowths from the streak were seen on the upper portion of the slant, where drying had begun; these were linear and usually rather short and could be noted with the naked eye as fuzziness at the edge of the growth. The appearance of these outgrowths was, however, by no means regular or constant in any of the four strains.

Fig. 9.—Microphotograph of culture No. 203 st., grown for seven days on Sabouraud's *media* plate, showing the typical mycelial outgrowth of the monilia and the grouped masses of conidia scattered along the mycelia. The entire field is shown in order to demonstrate the various planes in which the mycelia grow out, since they grow in the media and not on the surface.

At times the growth of No. 201 st. differed from the above description. The growth on the Sabouraud's *media* was not profuse nor yet scanty but considerably less than in strains No. 201 sk., No. 203 st., and No. 203 sk.; the growth was raised and colorless; at first translucent, later opaque. With the exception of these differences, the growth of No. 201 st. corresponded generally to the description of the other three given above. This particular strain developed, with time and after continued transplantation, a growth similar to or identical with the white, glistening opaque type which tended to replace the colorless translucent type. Possibly again this difference in

the appearance of the culture, like the difference in the appearance of the cell forms, was due principally to the varying rates of growth.

All the organisms grew well in a maltose-peptone-water media; both of the strains from patient I. S. (No. 203 st. and No. 203 sk.) showed a tendency toward bottom growth, no pellicle being formed; both of the strains from patient F. E. (No. 201 st. and No. 201 sk.) were top growers and formed a pellicle or thin scum which followed the fluid up the wall of the tube when the latter was wetted with the media. Our experience, however, with observations concerning top and bottom growth of organisms of this type, is such

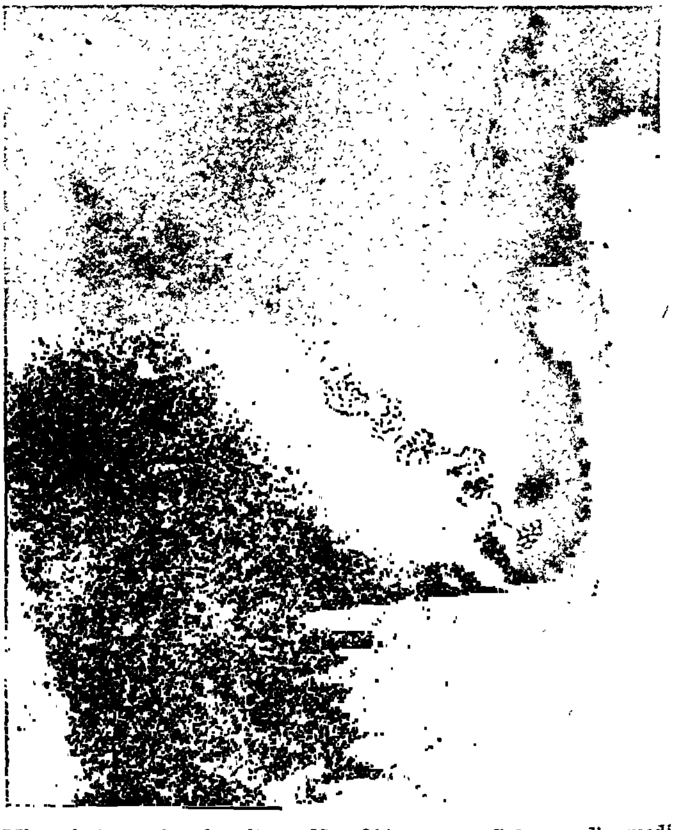

Fig. 10.—Microphotograph of culture No. 201 sk. on Sabouraud's media plate, seven days old, showing the mycelium connecting the grouped masses of conidia which are typical of the monilia.

that we are inclined not to lay too great stress upon this matter as a factor in differentiating these organisms.

Observations regarding morphology and the gross cultural characteristics of these organisms have given us but little aid in placing or identifying them biologically. The form of growth, their mode of reproduction is the only criterion to aid us in this direction. We have made cultures of the four organisms in thin pour plates, using the Sabouraud's agar medium. The plates were placed in a moist chamber at 37° C. In some cases the typical outgrowth occurred within forty-eight hours, again not before a week or ten days; in some cases all colonies showed the outgrowths, in others only a

single or a few scattered colonies showed the typical forms. At times none of the colonies showed the mycelial outgrowth, and only the yeast-like forms were present. In all four organisms at some time or another, however, one or more colonies showed the typical outgrowth described below.

The typical mycelial outgrowths are evident, first, as a few elliptical cells pushing out from the mass of the colony which otherwise is composed of round or elliptical yeast-like cells. One or several round or elliptical cells appear at the free end of this first cell and this process of additional cells appearing at the end of a cell may continue, and so a mycelium of varying length is produced. The length and thickness of these mycelial cells is quite

culture No. 201 sk. on Sabouraud's media, seven days g the typical grouped masses of conidia and an extension short oval cells, at the junction of two of which we find lls (conidia) which by budding in situ may later form down on the mycelium.

variable. They are not constantly elliptical but may be rectangular, with great exaggeration of the length; the width may be only half that of the usual oval or spherical cells. Occasionally they show branching, but this is not frequently noted. At times these mycelia are long—several times the length of the colony, or again they may be short, composed only of two or three cells. At the junction of these mycelial cells, usually, but not constantly, several round or oval cells appear forming rosettes. Usually the rosettes of cells increase by budding until we have at the junction points a mass of iregularly rounded or oval cells, giving the typical growth form. At

times these hyphae are thrust out into the media without the appearance of any rosette of cells at the junction points and in some cases the more typical form appears later (Figs. 9, 10, 11, 12).

At no times have we noted aerial hyphae, asci, spore sacs or any type or endospores. We have noted only the conidia (exospores) which form the mass of the growth.

Since no perfect forms of fructification are developed, these organisms, at least for the present, belong among the Fungi imperfecti, and according to the classification of Lindau in Engler-Prantl,[18] among the hyphomycetes. Further consideration of these classifications would place these organisms as monilia.

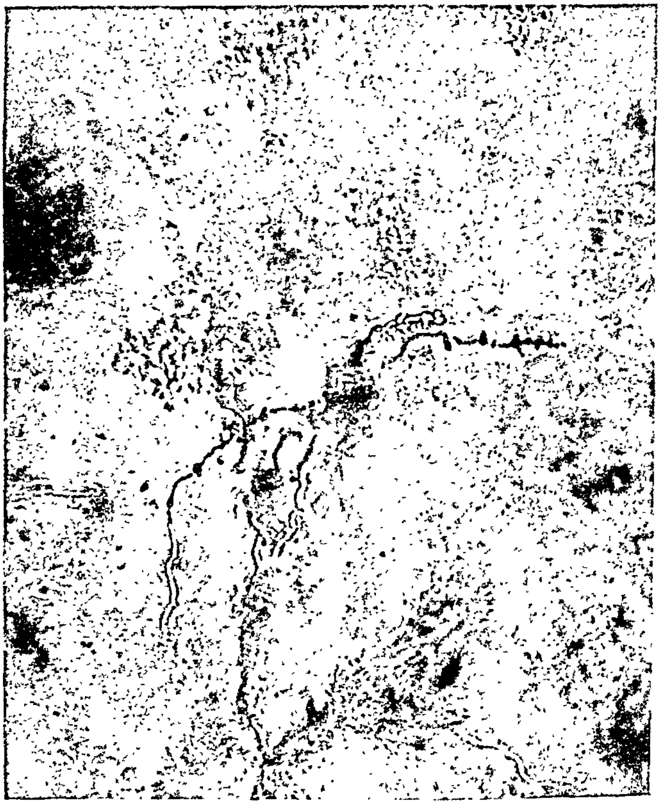

Fig. 12.—Microphotograph of culture No. 203 sk. on Sabouraud's media plate, seven days old, showing the simpler mycelial outgrowth with only a few conidia at the junction of two cells. These forms may later develop into the forms shown in Figs. 9 and 10.

Castellani and Chalmers[19] have attempted to classify the species of monilia on the basis of their action upon various carbohydrates and have, on this basis, differentiated a large number of species. The experience of one of us with a large number of strains of monilia (in as yet unreported work) and the experience of others makes it appear that this procedure is not satisfactory as a method for species differentiation. Castellani has himself noted that the monilia tend to lose with time (sometimes rapidly, sometimes more slowly) their power to ferment certain carbohydrates. We shall, therefore, not attempt to use the classification as outlined by Castellani and

Chalmers, but shall simply place these organisms in the genus monilia (Persoon).

We have, nevertheless, studied the actions of these four organisms on various carbohydrates, upon gelatin and milk, and Table I gives the results of this study. We wished principally to determine whether the two organisms isolated from the skin and feces of each case were identical or not.

It appears from this series of sixteen carbohydrates that the organisms isolated from the two patients are not identical organisms. The organisms from patient I. S. are distinctly more active in attacking the carbohydrates and in gas production than the organisms from patient F. E.

If we compare the actions upon carbohydrates of No. 201 sk. and No. 201 st., we find that these two organisms show the same actions upon all carbohydrates except upon xylose, melezitose and trehalose, and here the activity in fermentation varies irregularly. In comparing No. 203 sk. and No. 203 st. we find that these two differ only in their action upon xylose. We are inclined to believe that the differences noted between the two cultures from patient F. E. and the two from patient I. S. are negligible. Similar variations in the action upon the different carbohydrates have been noted a number of times in individual strains of monilia, when these were tested in the carbohydrates at various times after isolation.

We are, as a result of studies carried on in this laboratory, inclined to believe that the reactions of the monilia upon dextrose, levulose, maltose, saccharose and lactose are fairly constant and fixed qualities of the biologic activity of the various monilia; possibly the action upon inulin and dextrin is also quite definite. If the fermentation of carbohydrates is then to be used for purposes of differentiating monilia, we believe the first group mentioned above should be the only one relied upon for this purpose.

If we follow this scheme, it appears that the two organisms isolated from the skin and feces of patient F. E. are in all likelihood identical and the two isolated from patient I. S. are also probably identical, but the organisms from the two cases are apparently not identical.

Our studies then demonstrate the following facts: first, that monilia may be the causative agent of so-called Erosio interdigitalis; and second, that the organisms which appear in the dermatologic lesions may also be found in the feces of the affected individual.

DISCUSSION

The significance of the finding of the identical organism in the skin lesions and in the feces of these two cases may admit of two interpretations—either the occurrence and development of the organisms in the intestinal canal may have antedated the development of the skin lesions and the latter may be secondary to the intestinal infection, or the intestinal infection may have followed the dermatologic infection, and be due only to swallowing of the organisms from the hands, or swallowing materials contaminated through being handled by the patient; in which case we would consider the intestinal infection as simply chance and of little or no significance. It is, of course, impossible to state which of these explanations is correct, but it appears prob-

TABLE I

	DEXTROSE	LEVULOSE	MALTOSE	GALACTOSE	SACCHAROSE	INULIN	LACTOSE	DEXTRIN	ARABINOSE	XYLOSE	RHAMNOSE	RAFFINOSE	MANNITE	SALICIN	MELEZITOSE	TREHALOSE	GELATIN	MILK
F. E. Skin 201	AG	AG	AG	O	O	O	O	A	A	AG	O	O	O	O	A	AG	+	ALK cg
F. E. Stool 201	AG	AG	AG	O	O	O	O	A	A	O	O	O	O	O	O	O	+	ALK cg
I. S. Skin 203	AG	AG	AG	A	AG	O	O	AG	O	O	O	AG	AG	O	O	AG	+	ALK cg
I. S. Stool 203	AG	AG	AG	A	AG	O	O	AG	O	A	O	AG	AG	O	O	AG	+	ALK cg

A = acid production; G = gas production; O = neither acid nor gas production;

+ = growth along the line of stab but no arborescence; ALK = production of alkaline or no change of reaction; cg = coagulation of casein but no digestion (a soft coagulum is formed).

able, in view of the absence of general lesions and the absence of general effects in this type of infection, that the intestinal lodgment of the monilia is secondary to an initial infection of the skin.

If we consider our own findings and the findings of others concerning the organisms causing Erosio interdigitalis, it appears that in no case have blastomyces been demonstrated as the causative agents, since in no cases have cultures been obtained which show aerial hyphae. It is apparent, however, that ascomycetes have been demonstrated in some cases (by Hudelo, Sartory and Montlaur and Greenbaum and Klauder) and that Fungi imperfecti have been demonstrated in others—specifically endomyces, or botrytis and monilia,* these latter representing all rather closely related or similar organisms. It appears also that some of the unidentified organisms described by various observers may well be placed as either ascomycetes or as monilia, judging from the incomplete description given of the organism or the cultures obtained. With the present evidence then it appears that Erosio interdigitalis may be caused by either ascomycetes or Fungi imperfecti, especially members of the oosporae or the botrytidae.

It would appear further that the various names which have been applied to this type of dermatologic infection are not satisfactory or justified, in view of the types and the variety of etiologic agents, since they all carry the implication of either blastomyces or saccharomyces as the causative agents. If it is essential that the etiology be referred to, a term implying simply that fungi act as the causative agents would be justified.

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*Recently we have isolated a monilia from a third case of Erosio interdigitalis; this was an early case. It is questionable whether this third organism is identical with either of the two reported in this article.

CALCIUM DISTRIBUTION IN BLOOD*

By Paul L. Kirk and C. G. King, Pittsburgh, Pa.

It has long been recognized by the medical profession that sufferers from various types of jaundice often show a delayed blood coagulation time. This in many instances proves fatal following operations. Walters[1] reported that during the years 1918, 1919 and 1920, over 50 per cent of all patients operated upon with obstructive jaundice at the Mayo Clinic, died from intra-abdominal hemorrhage, or on necropsy, showed large amounts of blood in the intraperitoneal cavity. Analogous cases without jaundice during the same period, showed only 6 per cent fatalities from intraabdominal hemorrhage. This effect is presumably due to or parallel with, the presence of some biliary constituents in the blood. Lee and Vincent,[2] and Walters,[3] have shown that the intravenous injection of calcium chloride or lactate shortens the coagulation time, and lowers the proportion of fatalities following operation. Such a procedure is now widely used by physicians, but does not give uniformly satisfactory results.

It has been pointed out by Neuhausen and Pincus[4] that the inorganic constituents of the blood may be present in three forms: ions, undissociated molecules in equilibrium with the ions, and nonionizable compounds with some of the organic constituents. They have also shown that all the common inorganic constituents of pig serum are totally diffusible, with the exception of calcium. The nondiffusibility of part of the blood calcium has long been recognized, and variously reported as from 25 per cent to 50 per cent nondiffusible.

The methods used in studying the calcium distribution in blood have been divided into four general classes: (a) An attempt has been made to determine the concentration of the calcium ions[5] by electrometric methods, in which the value found was 20 per cent to 25 per cent. The method was unsatisfactory, and was stated to involve a possible error of ±25 per cent of the value found. (b) Dialysis has been used by various investigators. Clark[6] and later Loeb[7] used simple dialysis. Loeb's work showed that all the serum calcium was dialyzable in the presence of large quantities of salt solution. This would indicate that the colloidal complex may break down to yield diffusible calcium, if dialysis is carried out over a considerable length of time. Against distilled water his results were roughly in agreement with those of other investigators, as to the percentage of diffusible calcium. The development of compensation dialysis by Michaelis,[8] Rona,[9] and their stu-

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dents, and later used by von Meysenbug and his students,[10] is the only important advance made in this method. Von Meysenbug and his students found from 20 per cent to 40 per cent of the calcium to be nondiffusible, by his method of compensation dialysis. This work was carried out with extreme care, but all of the investigations that were made by this method are open to the criticism that a Donnan membrane equilibrium is established, and does not allow free dialysis of the inorganic constituents of the blood. Bayliss[11] has most clearly demonstrated the existence of such a permanent equilibrium. (c) Various forms of ultrafiltration through semipermeable membranes have been used. Cushny[12] made use of collodion sacs. Richter-Quittner[13] used a Zsigmondy-Bachmann filter. Later, Marshall and Vickers[14] used collodion sacs, and Neuhausen and Pincus[4] adopted their method. The quantity of serum used was comparatively large, about 30 c.c., and the outsides of the filters were not immersed in water. Moritz[15] objects to this procedure for small quantities of serum, because of the drying of the membrane, and the deposit of a film of serum on the filter. He used small collodion sacs, immersed in distilled water, and under a negative pressure not to exceed 180 mm. of mercury. (d) A method of determining that portion of the blood calcium which is active in producing the clot, based on the clotting time, has been used by Bauer and Narnickol,[16] and Vines.[17] The method consists essentially of the comparison of the clotting time of two portions of oxalated or citrated plasma, to one of which is added a calcium chloride solution, and to the other a portion of the blood being studied. Such a procedure gives little if any information bearing directly on the state of the blood calcium, since the assumption is made that the only factor involved is the calcium of the added blood.

Cameron and Moorhouse[18] have recently reported a study of the calcium distribution between dog's blood and the cerebrospinal fluid, and assumed that the total calcium content of the latter was the same as the diffusible calcium of the blood. The average value given on this basis was 53 per cent diffusible.

EXPERIMENTAL

A. *Methods.*—Of the four methods previously mentioned for the estimation of diffusible calcium in blood, the method of ultrafiltration under low pressures, seemed to be the least open to criticism, and was adopted for this investigation.

Most of the low pressure ultrafiltration has been done using negative pressure. Because of the high loss from evaporation of the filtrate, using this method, the use of positive pressure was adopted. The pressure employed approximated 150 mm. of mercury at all times. It was never allowed to go above 180 mm. or below 120 mm. of mercury. It has been pointed out by Neuhausen and Pincus[4] that between these pressures, there is no change in the character of the filtrate, and by previous investigators, that there is no precipitation of protein. It is also sufficient to induce a fair rate of filtration. The objections of Moritz, previously mentioned, were overcome in this method of ultrafiltration by filtering into an empty vessel which was practic

closed to the air by a loose joint, and which completely enclosed the collodion sac. We had no difficulty from drying of the filter, and no film was deposited on our filters except when exceptionally long periods of filtration were necessary. Our evaporation loss was checked for a period of ten hours, and was found to be about 0.02 to 0.03 c.c., which was considered negligible. We believe this technic to be an improvement over the methods described in the literature, because of its simplicity and dependability. Once started, our filters required no attention until filtration was complete. There was no variation in pressure, and the simplicity of our apparatus was the equal of any yet described.

Past investigations on blood by ultrafiltration have, in practically every case, been conducted on serum. Since this study was essentially concerned with the factors involved in coagulation, it was considered preferable to use plasma, because it had undergone no changes from coagulation. The difficulty encountered here was in obtaining an anticoagulant which did not affect the calcium. Hirudin, or the extract of the medicinal leech, was decided on, as being the most suitable anticoagulant, following the suggestion of Richter-Quittner.[19] It has been shown that this material acts as an antithrombin, and has no action on the mineral constituents, whereas, oxalates, citrates and fluorides either precipitate or bind the calcium.[20, 21, 22] It has likewise been shown that these materials affect the P_H of the blood.[23] Some difficulty was encountered in obtaining this extract, and the first portion of this study was consequently made on serum. Later, leeches were purchased, and extracted in our own laboratory. This procedure was not entirely satisfactory, due to the difficulty of standardization of the extract, and its tendency to undergo rapid bacterial decomposition. The material was repeatedly pasteurized, and kept in the ice box. Its action was satisfactory enough of the time to give significant results, however.

The blood calcium was determined by the method of Kramer and Howland.[24] This method, while somewhat more laborious, gives more satisfactory results than the methods of Lyman,[25] or of Kramer and Tisdale.[26] An analysis was made on the plasma or serum of the original sample. A duplicate analysis was made on the filtrate, after the ultrafiltration. All analyses were made in an identical manner, so that the results are comparable, even though they may not represent absolute values. Checks were run whenever the size of the sample permitted it.

All ultrafiltration, and handling of the blood previous to ultrafiltration, was carried out as nearly as possible at 37° C. For this purpose, samples were received in warm tubes, carried in small Dewar flasks, centrifuged in warm cups, and ultrafiltered in an incubator at 37° C. immediately. Our experience indicates that it is preferable to work at lower temperatures. A very few hours at this temperature is sufficient to induce vigorous bacterial growth in case of accidental contamination. Moreover, it has been shown by Moritz[15] that there is a progressive increase in the percentage of diffusible calcium, in blood standing at room temperature.

No check on the P_H of the blood was made. It has been shown by Menten,[23] however, that hirudin added to blood does not affect to any extent the hydro-

gen-ion concentration. In general, no other treatment was used which would affect the P_H, and the blood was not allowed to stand for long periods of time.

In practically every case the ultrafiltrates were tested for protein, either by the biuret reaction, or by the xanthoproteic test, or both, and in no case was a positive protein test obtained. All jaundiced blood was tested for the presence of bilirubin, by the method described by Kapsinow,[27] using Obermayer reagent. The results were entirely satisfactory. Many of the ultrafiltrates were similarly tested, and no positive tests were given. After filtration, the deep color of the filters also showed the retention of bile pigment.

B. *Apparatus.*—The ultrafilters consisted of collodion sacs, fastened to test tubes, the bottoms of which had been removed. The collodion was made from a high viscosity nitrocellulose, 10 g. of which was dissolved in a mixture of 50 c.c. absolute ether, 50 c.c. absolute alcohol, and 10 c.c. glacial acetic acid. Olive oil was substituted for the acetic acid in preliminary experiments, following the suggestion of Moritz,[15] but the resulting sacs were not as strong as those made with the acetic acid. The use of glacial acetic acid in this manner has been recommended by Eggerth.[28] The sacs were made by filling tubes with collodion, centrifuging for two minutes and draining. This was repeated a second time. After the second draining, the tubes were dried with constant rotation for ten minutes. They were then immersed in water and the sacs could be easily removed in about ten minutes, by holding them under water and loosening them in the tubes. The tubes were of such size that the sacs fit tightly on the outside of test tubes. The bottoms were cut from the test tubes, the sacs slipped on, and bound there by a firm wrapping with silk thread. No difficulty was experienced from leakage or loosening of the sac.

Large test tubes were melted down at the open end, to such a size that the filter tube would just enter, but so that the flare on top of the filter tube would fit snugly. These large tubes served as receiving vessels for the filtrate.

The apparatus used for application of pressure to the ultrafilters, consisted of a five gallon carboy to which suitable connections were made with pressure lines. The carboy was stoppered with a three-hole rubber stopper, which was wired in tightly, and covered with sealing wax. Through one hole was placed a tube connected with a stopcock on the water line. This tube extended to the bottom of the carboy, and served as a siphon for emptying the vessel. Through a second hole was placed an open manometer, calibrated for 120 mm., 150 mm., and 180 mm. Through the third hole was connected a line to the ultrafilters in the incubator. In this line was included a three-way stopcock, one branch of which opened to the air, to allow release of pressure, and also the intake of air on siphoning out the water from the carboys. The end of this line was branched, with a stopcock on each branch and the ends of the branches were fitted with small, flexible, rubber stoppers, which exactly fit the ultrafilter tubes. All lines were constructed

entirely of glass and pressure tubing, with as little of the rubber tubing as was practical. All joints in the apparatus were wired, and trebly shellacked. No difficulty with leakage was encountered. The pressure was obtained by displacement of air in the carboy by water.

The apparatus used for the calcium determinations followed closely the description in the original article of Kramer and Howland.[24] The titrations were made entirely with 10 c.c. burettes, calibrated in twentieths of a cubic centimeter. For ashing the blood, both platinum crucibles and Vitreosil crucibles were used. No previous record has been made of the use of Vitreosil for this purpose. Some difficulty was experienced with the Vitreosil. The results seemed to run lower in general than in platinum, on the same samples. A deposit of crust was formed by the ash in the Vitreosil crucibles, which did not readily respond to treatment with dilute acids, so that these crucibles were replaced entirely with platinum. All ignitions were made over Meker burners.

C. *Experimental Data.*—

1. *Normal Blood.* Blood was taken in most cases from hospital patients whose disorders were such that they would not be expected to affect the calcium distribution. A few were taken from patients in which the calcium was found to be definitely affected. These will be included under normal blood, signifying merely that they showed no jaundice. The concentration of calcium found is in every case expressed in milligrams per 100 c.c. of plasma or serum.

CASE 1.—Middle-aged, colored, male, with gastric disorder. Blood slightly clotted during filtration.

Total Ca, 8.49; ultrafilterable Ca, 67.4 per cent.

CASE 2.—Middle-aged male with some hypertension. Blood clotted slightly during filtration.

Total Ca, 7.58; ultrafilterable Ca, 67.7 per cent.

CASE 3.—Middle-aged male with gastric disorder. The sample was small which greatly increased the possibility of error.

Total Ca, 9.24; ultrafilterable Ca, 80.9 per cent.

CASE 4.—Male, age twenty-three. No abnormal conditions.

Total Ca, 7.43; ultrafilterable Ca, 72.3 per cent.

CASE 5.—Female, early middle age, a short time after induced abortion.

Total Ca, 7.69; ultrafilterable Ca, 56.0 per cent.

This low value can be correlated with the effect of pregnancy, as will be indicated by the next two cases.

CASE 6.—Female, about twenty-five years of age, in late pregnancy. Filtration made on serum.

Total Ca, 12.51; ultrafilterable Ca, 26.85 per cent.

CASE 7.—Middle-aged female in late pregnancy.

Total Ca, 13.94; ultrafilterable Ca (serum), 16.87 per cent.

2. *Jaundiced Blood.* Some of the samples of jaundiced blood were allowed to clot, and serum runs made. Others clotted due to insufficient hirudin, while some were made on plasma.

CASE 1.—Middle-aged colored man, slightly anemic, jaundiced, due to cirrhosis of the liver, caused by acute syphilis. Treatment was being given in the form of neoarsphenamine injections. Icterus index was 50 to 60.

Total Ca, 9.80; ultrafilterable Ca, 45.95 per cent.

CASE 2.—Female, late middle age, slightly jaundiced. Cholelithotomy performed one month previously. The original icterus index was 20. At the time of drawing sample it was 10.7. The patient was recovering from marked anemia.

Total Ca, 11.39; ultrafilterable Ca (serum), 55.1 per cent.

CASE 3.—Female, middle age, very slightly jaundiced from obstruction of the common bile duct.

Total Ca, 7.86; ultrafilterable Ca, 66.5 per cent.

CASE 4.—Female, seventy-two years of age, obstructive jaundice, highly developed.

Total Ca, 4.31; ultrafilterable Ca (serum), 50.6 per cent.

CASE 5.—Male, late middle age, slightly anemic, slightly jaundiced due to obstruction. Clotting time, 10+ minutes.

Total Ca, 6.94; ultrafilterable Ca (serum), 59.9 per cent.

CASE 6.—Female, late middle age, previously jaundiced four times. Not jaundiced at time of drawing sample. Diagnosed as having gallstones.

Total Ca, 7.91; ultrafilterable Ca, 64 per cent.

CASE 7.—Female, middle age, highly jaundiced due to obstruction. Coagulation time, 6.5 minutes.

Total Ca, 8.52; ultrafilterable Ca (serum), 48.2 per cent.

3. *Other Pathologic Conditions.* One case only of advanced nephritis and uremia was obtained.

CASE 1.—Male, late middle age, advanced nephritis and uremia. Blood clotted somewhat due to insufficiency of hirudin.

Total Ca, 7.49; ultrafilterable Ca, 51.15 per cent.

4. *Anticoagulants.* Dog's blood was used in this study. All samples were drawn at the same time from an artery, while the animal was anesthetized by ether. The anticoagulants used were hirudin, sodium citrate, potassium oxalate, and sodium fluoride. The total calcium of the blood plasma was 8.68 mg. per 100 c.c.

ULTRAFILTERABLE CALCIUM

	Hirudin	Sodium citrate	Potassium oxalate	Sodium fluoride
First Series	72.7 per cent	86.2 per cent	0 per cent	13.98 per cent
Second Series	69.7 per cent	100.0 per cent	0 per cent	0. per cent

The second series of samples were kept for twenty-four hours in the ice box. The second sample treated with hirudin clotted, and the serum was filtered. The first series was run immediately after drawing.

5. *Biliary Constituents.* The only biliary constituents which were tested for their effect on the blood calcium distribution were sodium taurocholate and sodium glycocholate. These were mixed in equal portions, and 0.26 g. was added to 20 c.c. of normal human blood, treated with hirudin. This may have affected the P_H of the blood to some extent.

Total Ca, 7.43; ultrafilterable Ca, with bile salts, 55.2 per cent; ultrafilterable Ca, normal, 72.3 per cent.

D. *Discussion.*—Since only a limited number of jaundice cases were available during the period of this investigation, and since considerable difficulty was encountered in obtaining blood which could be considered as normal in respect to the calcium distribution, it is considered dangerous to make too sweeping generalizations. It appears, however, that the percentage of diffusible calcium is somewhat lower in jaundiced than in normal blood, and this lowering is roughly proportional to the severity of the jaundice. Since there are several other conditions in which the percentage of diffusible calcium is considerably lower than in the normal adult, without any marked decrease in the coagulability of the blood, it appears probable that the lowering of the diffusible calcium in jaundice is not the only factor involved in increasing the coagulation time, if it is involved at all. Schultz[29] has shown that platelet deficiency is a factor in the diminished coagulability of the blood in anaphylaxis, and we plan to investigate this factor in jaundice in the near future.

Another factor which has not been sufficiently studied in regard to diffusible calcium, is the amount and kind of protein present in the blood in jaundice. Loeb and Nichols[30] have shown that the calcium is bound to certain types of blood proteins. Perhaps there are other types which do not bind calcium, and a disturbance in this protein balance might affect the degree of diffusibility of the calcium.

Normal Cases 6 and 7 indicate that pregnancy probably has a very considerable effect on the distribution of the blood calcium. Total calcium seems to be high and ultrafilterable calcium to be abnormally low. This does not agree with the results of Cameron and Moorhouse.[18]

A comparison of the results obtained upon plasma and serum indicate that very little if any difference may be expected so far as the ultrafilterable calcium is concerned.

It was found that the common anticoagulants should not be used in studies on blood calcium distribution. Oxalate eliminates all the ultrafilterable calcium in a short time. Fluoride is slower in action, but on standing, puts all the calcium in a nondiffusible form. Citrate, however, slowly increases the diffusible calcium. Probably the removal of the calcium ions to form a citrate-calcium complex displaces the equilibrium between the ionic and colloidal calcium, with removal of ions and further formation of the diffusible citrate-calcium complex. This action is rather slow, as is also the action in long dialysis of blood against salt solution,[7] indicating that the calcium equilibrium is only very slowly displaced.

SUMMARY

The total and ultrafilterable calcium has been estimated in the blood of seven persons showing evidence of a jaundiced condition.

Comparison with normal cases indicates that the percentage of ultrafilterable calcium found in this condition is below normal. The difference is not thought to be sufficiently great, however, to account for the frequent delayed coagulation.

Preliminary indication is given that the ultrafilterable calcium is low during pregnancy.

The use of citrate, oxalate, and fluoride as anticoagulants were found to cause a very marked change in the calcium distribution in blood.

An improvement in the procedure for studying the ultrafilterable constituents in blood has been developed.

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CYTOMORPHOSIS OF THE TUBERCLE BACILLUS AND OTHER ACID-FAST MICROORGANISMS*

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THE tubercle bacillus, on account of its economic importance as a disease-producing microorganism, has been studied probably as exhaustively as any one other low form of life, and yet, from a practical standpoint, there are still many logical questions which arise involving the bacillus itself which have only been touched upon superficially and inconclusively. In explanation of this may be taken the slow growth of this microorganism and its polymorphic nature both in the culture tube and in the body. That there is a relation between the morphology and the character has been suggested a number of times, but all efforts to correlate the two, especially in the human or animal economy, has been of no avail. This assumes importance, especially when it is realized that our present day clinical determinatives of the activity of tuberculosis take into consideration only the body tissue or cellular reaction for the purpose of interpretation, and the bacillary factor is dispensed with in the simple terms negative or positive. Thus also our experimental efforts leave much to be desired from the standpoint of bacillary interpretation as to the effect and value of certain therapeutic attempts. Irregularity in length, thickness, contour, and form (as well as in mode of division) is the characteristic of the tubercle bacilli and other acid-fast microorganisms, according to Miehe,[1] who studied the morphology and systematic classification of the tubercle bacillus. The tubercle bacillus according to him is an amycelial mold like yeast, the unit being the cell and not the mycelium. It belongs to the family mycobacteriaceae and is not a degenerated parasitic form of the saprophytic molds found in nature, as suggested by Coppen-Jones,[2] Bruns and others.

In scanning the literature, and after having examined numerous microscopic specimens, both from cultures of tubercle bacilli and from animal or human tuberculosis, one is impressed with certain striking morphologic characteristics which merit consideration in following the consecutive changes undergone by the tubercle bacillus in the culture tube or in the body. The long filamentous and branching types have excited intense interest, especially among investigators interested in the classification of this microorganism. The majority of reports record these forms especially in avian cultures, and in mammalian cultures grown under abnormal conditions. They have also been recorded as occurring in certain positive sputums.[3] Intermediate to these long filamentous branching forms are the usual longer and shorter straight and curved forms commonly occurring in the average well-grown

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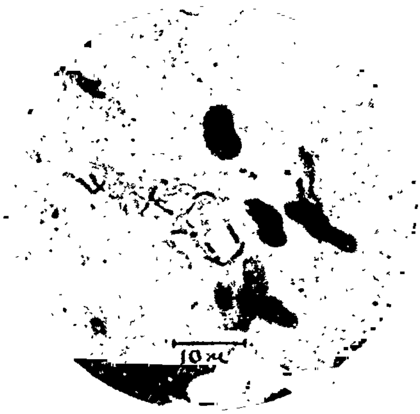
culture and seen in the ordinary sputum from open cases of pulmonary tuberculosis.

In 1905 Carl Spengler[4] emphasized a smaller acid-fast form which he termed "Splitter" and which has led to a great deal of discussion and probably had much to do with the identification of Much's granules. Practically neither the "Splitter" nor "Much Gram granules" have proved of value[5] but the "Splitter" are of interest here because of their specific acid-fast character. Spengler utilized only groups of "Splitter" for diagnostic purposes and describes them as being acid-fast clusters or granules. He considered them involution forms of the tubercle bacillus of reduced vitality and virulence. Their resistance to chemicals is less than that of the ordinary rod forms. They are found in cultures only where the nutrition is reduced and unfavorable to the bacilli. They occur as a rule with bovine bacilli when the nutrient medium is unfavorable and not so frequently with human bacilli. Whether these "Splitter" are in any way related to the more deeply-staining segments occurring normally in many of the bacilli is not elucidated.

In an ingenious experiment Levy[6] succeeded in growing human tubercle bacilli at temperatures of 41° and 42.5° C. but did not succeed by this means in altering the morphology of the cultures as compared to those grown at 37° C. In reviewing the subject of the existence of spores he points out that there exist in tubercle bacilli doubly refractile bodies which take a deep, dark-red fuchsin stain and resist decolorizing with mineral acids. If these are spores or "resting bodies" they do not conform to the classical definition with regard to the resistance to heat, etc. Szepesi,[7] after staining tubercle bacilli by means of carbolfuchsin, impregnated them with silver and found that the bacilli did not uniformly take up the silver nitrate but black granules appeared in certain locations, either at the ends or in the middle as 1, 2 or 3 (never more) sharply marked off granules. It is believed that the granules consist of a combination of silver and protein, since they dissolve in potassium cyanide solution. They are not the same as the Gram granules which are supposed to consist of neutral fats, according to Much. The silver granules seem to differ only slightly from the polar bodies seen in Ziehl preparations and whether they are spores or not remains undetermined. Similar structures were not demonstrable in staphylococci, typhoid and paratyphoid bacilli.

Probably the most exhaustive and elaborate study of the changes occurring in human and bovine tubercle bacilli in culture is that reported by Wolbach and Ernst.[8] They studied four human and seven bovine strains of tubercle bacilli at various intervals during cultivation up to three to four weeks. In summarizing their results with the cultures of human and bovine origin, they state that during the first days of growth the form assumed by the tubercle bacillus is longer than that of the later stages, and these forms grow progressively shorter until the type form of the fully developed culture is reached. The irregularly stained and branched forms occur at a period of vigorous multiplication, and cannot be considered as involution or degenerate

forms. Neither the age of the culture used nor the medium on which it was grown affected the growth or microscopic appearances of the subcultures on egg. Likewise the reaction of the egg medium had no effect on the general characteristics and changes occurring at different ages. The changes described occurred with all the cultures used, though requiring different lengths



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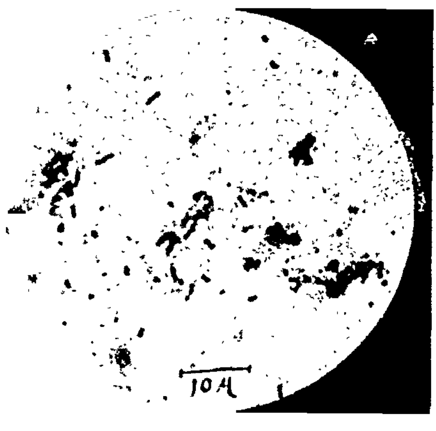

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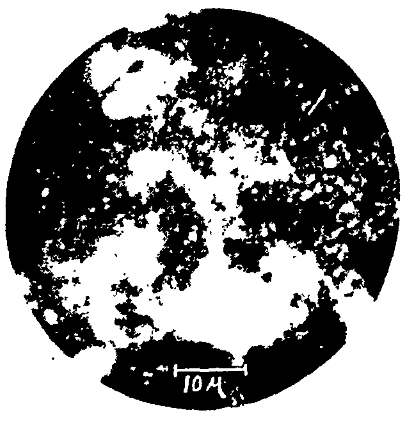

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Photomicrograph Series A.

Cytomorphosis of "Koch-Novy" tubercle bacilli grown on glycerol agar at 37° C.

1. Appearance of bacilli after incubation for one day. Acid-fast fuchsin-stained bacilli appear as black rods, nonacid-fast methylene-blue-stained bacilli appear as faint gray shadows.

2. Appearance of bacilli after incubation for one week.

3. Appearance of bacilli after incubation for one month.

4. Acid-fast fuchsin stained lipoid globules found after nineteen months at room temperature following one week at incubator temperature.

of time for their completion, varying from fourteen to twenty-six days. The greatest variations in form and staining reaction were found in rapidly growing cultures and they agree with Coppen-Jones that a favorable medium and free access of oxygen are best suited for the production of branched and filiform forms. The only interpretation of the great diversity of form assumed

by the tubercle bacillus when grown under most favorable conditions is that it is truly pleomorphic and should be classed among the higher bacteria.

It is only natural that the question of the changes occurring in tubercle bacilli in the culture tube and in the body should involve the problem of bacteriolysis, and Isabolinsky and Gitowitsch[9] recently report that the tubercle bacillus undergoes biologic changes when grown upon the liquid nutrient egg medium of Besredka which are viewed as bacteriolysis. After six weeks' incubation at 38° C. the number of Ziehl staining morphologically unchanged bacilli become fewer and the Much-Weiss forms increase. Under the influence of lipoid-containing substances, tubercle bacilli undergo all stages of lipolysis, from loss of acid-fastness to complete solution. The best results occurred with lecithin, olive oil and green soap. Sodium and potassium salts, as well as alkalies, possess no bacteriolytic properties.

The apparent hopelessness of utilizing the information on the tubercle bacillus at present at hand for clinical interpretation is voiced by Sorley,[10] who elaborately reviews the literature and reports the results of examination of sputums with a view to an interpretation of the morphology of the bacilli, the presence of "Splitter" and of Much granules. He concludes that "the presence of the granules implies simply that the bacillus is in a state of active reproduction, the presence of interpolar granules that the bacillus has attained a stage of greater or lesser maturity, and that, whether the bacilli be few or many, the severity of the disease and the prognosis are not necessarily affected thereby." Georgevitch[11] likewise sees no relation between the clinical evolution and the number of bacilli in the sputum or their morphology.

In 1923 Corper and Gauss[12] reported the results of a study of the viability of tubercle bacilli on culture mediums. It was found that fully grown cultures of human tubercle bacilli may remain viable on gentian-violet egg medium or 5 per cent glycerol-agar, whether kept in the ice box or in the incubator, for about four to eight months, so that successful transplants can be obtained on these mediums up to this time. Bovine tubercle bacilli are more resistant on these mediums and may remain viable (in the sense of furnishing subcultures) for from about eight to sixteen months (the latter in exceptional cases). The more vigorous and profuse growers seem to give successful transplants after longer intervals than the less vigorous. At that time it was also pointed out a culture may still contain sufficient viable bacilli to infect a highly susceptible animal like the guinea pig, when injected in large amounts, after nineteen months and still will not grow on artificial mediums (Theobald Smith).

In elaboration of these studies and on the basis of the information available as a result of the recent excellent contributions by Henrici[13] on bacterial cytomorphosis, it seemed advisable to study the changes that occur during the growth and loss of viability of the tubercle bacillus in the culture tube and if possible to correlate this with the morphologic changes noted in the animal body, giving careful attention to the length and staining properties of the tubercle bacilli. Since it is the acid-fastness that specifically characterizes the tubercle bacillus, none of the other nonacid-fast staining methods were utilized in

this preliminary study. Bacteriologists have most generally accepted the teachings that the morphologic characters of bacteria are invariable except for such pathologic changes as may be brought about by an unfavorable environment, with a minority believing that they exhibit a complex "life cycle" similar to that of protozoa or higher fungi, the various stages of which may be found only in varying mediums (Löhnis). The error in both these teachings is due to an insufficient consideration of the element of time and Henrici, as a result of quantitative studies of the morphologic characters of several species of bacteria, has arrived at a theory differing from either of the aforementioned. It is, briefly, that the cells of bacteria undergo a regular metamorphosis during the growth of a culture similar to the metamorphosis exhibited by the cells of a multicellular organism during its development, each species presenting three types of cells, a young form, an adult form, and a senescent form; and that these variations are dependent on the metabolic rate, the change from one type to another occurring at the points of inflection in the growth curve. The young or embryonic type is maintained during the period of accelerating growth, the adult form appears with the phase of negative acceleration, and the senescent cells develop at the beginning of the death phase. The term "involution form" implies changes associated with senescence and death. Increase in size of young growing cells may be six times that of resting cells and is accompanied by other changes in morphology such as disappearance of intracellular granules, the protoplasm becoming more hyaline and staining more deeply. Young cultures may also be more susceptible to harmful agents than are older ones, a parallel with multicellular organisms.

It is pointed out by Henrici[14] that he found the diphtheroid group as an exception, decreasing in size during the period of active growth in agreement with Clark and Ruehl's[15] findings. V. Cholera[16] with increasing rapid growth assumes a long, plump, relatively straight form; with decreasing growth the cells become more slender and curved (the typical "vibrio" form), and when growth is arrested and death begins the cells assume bizarre forms showing bulgings and "budding" and many become spherical. It is noteworthy that only the mature cells are spirella, the embryonic forms being bacilli and the senescent type cocci.

In order to obtain as much information as possible which might elucidate the problem of the changes occurring in the tubercle bacillus in the body, the studies to be recorded below were planned so that the morphologic changes occurring during the growth of not only tubercle bacilli but other closely related acid-fast organisms in the culture tube were studied. The more rapid growth and apparently larger size of the latter would lend itself better to more detailed microscopic examination as well as covering possible greater changes in morphology over a shorter time period. This phase of the study would also serve to note any differences between the more rapid growers and genuine tubercle bacilli, if such existed. The four strains of rapid growers studied were a Smegma bacillus, an Avian tubercle bacillus, an acid-fast organism "Day," and a rapidly growing avirulent strain of tubercle

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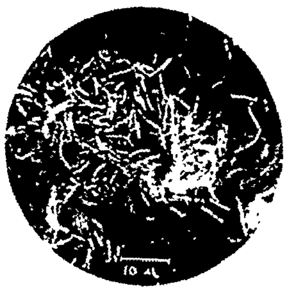

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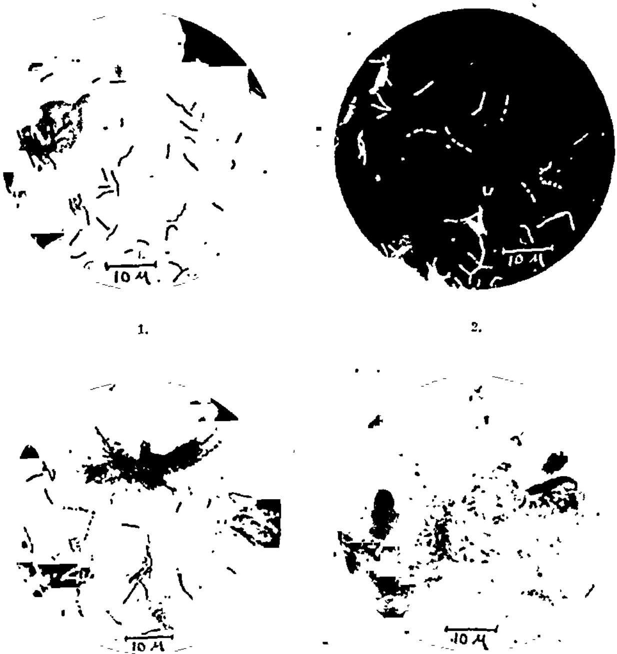
Photomicrograph Series E.

Cytomorphosis of "Day" tubercle bacilli grown on glycerol agar at 37° C.
1. Appearance of bacilli after incubation for three days.
2. Appearance of bacilli after incubation for one week.
3. Appearance of bacilli after incubation for one month
4. Appearance of bacilli after incubation for two months.
5. Appearance of bacilli after incubation for four months.

bacilli ("Koch-Novy"). The latter was brought from Koch's laboratory in 1888 by Dr. F. G. Novy of the University of Michigan when it was still virulent,[17] while the former three strains were obtained from Dr. E. R. Long of the University of Chicago several years ago and have been maintained by weekly transfers onto new mediums. In our hands these strains gave profuse cultures in the incubator on glycerol-agar in one to two days. In this study no attempt was made to grow them on different mediums but young cultures one to two days old were transferred onto a series of 5 per cent glycerol-agar tubes which were then capped by paraffined cotton plugs and paraffined cloth to prevent drying out of the mediums. Smears were made from one of the tubes after incubation for one, two, three, and four days, one and two weeks, one, two, and four months, no tube being used twice for obtaining smears, thus avoiding the possibility of changes occurring incident to the introduction of a contaminator. The smears were stained after fixing, by means of steaming carbolfuchsin for thirty seconds, washed with water, decolorized with acid alcohol, and counterstained with methylene-blue for thirty seconds. Since it is almost impossible to obtain uniform direct smears from cultures, the bacilli were first ground up in a centrifuge tube and a uniform suspension made in saline solution to which there was added an equal volume of acetone and from which the smears were made. In general there was noted in the microscopic examination of the smears a marked variation in size and beading of the bacilli and only by a rather approximate quantitative examination of a large number of bacilli could deductions of any value be drawn. When, however, a series of slides were examined which had been taken at different intervals, there was a striking contrast between the size and shape of the bacilli as well as in the acid-fast beading or granules noted in the bacilli. In all the rapidly growing cultures the findings in this respect were practically uniform and they, therefore, can be reported in brief together. Minor individual variations occurred but, for the solution of the problem at hand, were of no especial significance. Of the variables, however, it was rather interesting that only one of the rapidly growing forms, the Koch-Novy strain, reputed to have originated from a tubercle bacillus, in the first days of cultivation revealed a large percentage of nonacid-fast bacilli on the glycerol-agar, by the staining technic used. This did not occur with the three other rapid growers. Uniformly, however, the four strains of acid-fast bacilli revealed during the first few days of growth quite a large number of long (up to 8 to 10 microns) filamentous straight and slightly curved bacilli interspersed with the usual shorter straight and curved bacilli uniformly fuchsin stained. At this time beading was to a great extent absent, although an occasional beaded and short bacillary form was seen. After one week's growth the long forms still persisted but beading, irregular and in streptococcus- or streptobacillus-like chains, began to appear. The striking forms at this time were the long strings of acid-fast beads in which the beads were a deep red while the intermediate parts were free from stain. The long forms of uniformly stained bacilli still were found at this period but the number was markedly decreased over the earlier periods. After one month the uni-

formly stained long forms were practically absent and there were a large number of chainlike beaded forms and short cocco-bacillary forms began to appear. In the Koch-Novy strain the cocco-bacillary forms predominated at the one-month interval, the changes in this organism having run a more rapid course than with the other forms of rapidly growing acid-fast microorganisms in which the coccus and short bacillary forms began to predominate only



1. 2.

3. 4.

Photomicrograph Series C.

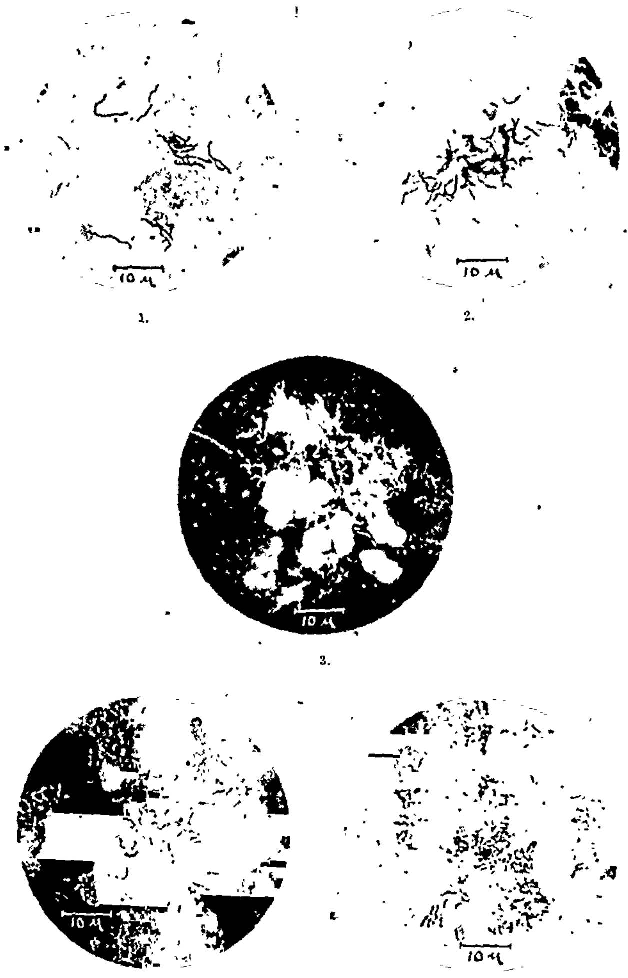
Cytomorphosis of Smegma bacilli grown on glycerol agar at 37° C.
1. Appearance of bacilli after incubation for one day.
2. Appearance of bacilli after incubation for one week.
3. Appearance of bacilli after incubation for one month.
4. Appearance of bacilli after incubation for four months.

after two and four months' cultivation at 37° C. During the course of this study, cultures were also examined which had been incubated at 37° C. for one week and then had been kept at room temperature in a dark box for two years. Many of these, although showing macroscopic culture, revealed upon

microscopic examination only a few coccus forms and large numbers of acid-fast globules. These various changes are well depicted in the appended illustrations.

In the study of the pathogenic varieties of tubercle bacilli, there were included two human and two bovine strains, all slow-growing cultures but differing in virulence for the guinea pig. One strain "Human" was a relatively avirulent strain of human tubercle bacilli producing a generalized tuberculosis in the guinea pig by injection only in amounts exceeding 1 to 10 milligrams and growing rather sparsely at 37° C. on glycerol-agar and egg mediums within two months. The other human strain "Gluckson" was a highly virulent organism producing a generalized disease in the guinea pig when injected in amounts as low as 0.000,000,001 milligram and growing profusely at 37° C. on glycerol-agar and egg mediums in two months. Both bovine strains ("Bov. D." and "Bov. Vir.") grew profusely on glycerol-agar and egg mediums within two months, and one ("Bov. Vir.") was highly virulent to guinea pigs, infecting in amounts less than 0.000,001 milligram, while the other ("Bov. D.") was moderately virulent. Since there occurred barely an appreciable growth with these strains before three weeks at 37° C., the first smears were made from cultures on glycerol-agar and Petroff's gentian-violet egg medium at this interval and after six weeks, three months, six months, and nine or ten months. All culture tubes were seeded from fairly well-grown parent cultures about one to two months old. Suspensions were made in saline solution and smears prepared as described for the rapidly growing bacilli above.

As a whole, except for the slow and gradual course of the changes, the same morphologic transformations with age occurred in the cultures of the slow-growing tubercle bacilli as were seen with the rapidly growing acid-fast forms. The less profuse growing "Human" culture did not reveal the long bacillary forms as seen in the cultures of the more profuse growing human strain "Gluckson" or the bovine strains but otherwise there was found a fair uniformity in the changes as they occurred with prolonged cultivation up to ten months. The long filamentous uniformly staining bacilli (in length up to 10 microns) predominated in the early periods of cultivation up to six weeks to three months, after which the shorter forms (3 to 5 microns) appeared and finally, at nine to ten months, the short bacillary (1 to 3 microns) and coccoid acid-fast forms predominated. It is noteworthy that in old glycerol broth cultures of tubercle bacilli which had remained in the incubator at 37° C. for as long as four years, short bacillary and coccoid acid-fast forms still persisted. It is interesting also that, although fewer in number at any certain period of examination, the length (8 to 10 microns) of some of the bacilli from the virulent human strain "Gluckson" on glycerol-agar or egg medium equalled that seen for the rapidly growing forms on the same mediums, making it seem reasonable that the rate of growth was accountable for the greater preponderance of the long forms at any one time during the early periods of growth in the rapidly growing culture.



Photomicrograph Series D.

Cytomorphosis of virulent human tubercle bacilli ("Gluckson") grown on glycerol agar or gentian-violet egg medium at 37° C.

1. Appearance of bacilli after incubation for three weeks.
2. Appearance of bacilli after incubation for six weeks.
3. Appearance of bacilli after incubation for three months.
4. Appearance of bacilli after incubation for six months.
5. Appearance of bacilli after incubation for ten months.

No consecutive sequence of the appearance of polar bodies could be definitely determined in the foregoing culture studies, although it seemed that they predominated especially during the intermediate periods of growth, occurring not in the long filamentous uniformly staining forms nor in the coccoid or short cocco-bacillary forms. The irregularity of their appearance made exact determinations especially difficult, and further study, probably by means of special specific staining methods, is required to attach to them a definite significance. Unlike spores in spore-forming bacteria, the tendency of the tubercle bacilli seemed to be rather to the formation of the shorter uniformly stained bacillary and coccoid forms from which polar bodies were lacking.

Having thus noted a definite sequence of morphologic growth changes in the rapidly growing acid-fast microorganisms studied which resemble in the main those occurring in the genuine human and bovine tubercle bacilli growing slowly on culture mediums, the second part of this study was concerned with the correlation of the culture studies to the changes occurring in human and bovine tubercle bacilli in the animal body. For this purpose the same two human and two bovine strains of tubercle bacilli were injected subcutaneously into guinea pigs. The experimental details will not be presented here except in brief, as they serve no particular end and add nothing to the clarity of the presentation of the problem. For the sake of uniformity, all the cultures used had been grown for six weeks at 37° C., the period when most active and profuse growth had attained. Stained smears were prepared from all cultures used for inoculation at the time of inoculation to serve as controls for the changes occurring in the body. Bacilli from both glycerol-agar or Petroff's gentian-violet egg medium were injected subcutaneously into normal guinea pigs in amounts ranging from 0.1 to 100 mg. of a uniform suspension in 0.5 to 2 c.c. of 0.9 per cent sodium chloride solution for the purpose of obtaining as wide a variation as possible in the concentration of bacilli in the tissues so that no morphologic variations would be missed in the study. In addition the same suspensions used in viable form were subjected to 100° C. for thirty minutes to kill the bacilli, and were then injected subcutaneously in amounts ranging from 50 to 200 mg. after stained smears had been prepared for comparison with smears of living suspensions and for comparison with the stained smears prepared subsequently from the pus obtained from the guinea pigs. Thus it was hoped it would be possible to note any morphologic changes occurring in heat-killed bacilli capable of producing only local tuberculous changes in the animal body and compare them with the changes occurring with viable avirulent and virulent human and bovine tubercle bacilli, the latter being capable of multiplication at different rates in the body and producing a generalized disease with fatal termination in the case of the virulent forms within a short period (one to two months) of time. In those cases in which pus developed and it was possible to obtain sufficient for the purpose, stained smears were prepared five, eight, eleven, twenty, thirty, fifty and ninety days after subcutaneous injection of the suspension of the bacilli. The staining technic

followed throughout this series was identical and uniform in order to rule out possible errors from this source. Likewise smears were made as nearly uniform as possible. The majority of the guinea pigs receiving the injections of the highly virulent strains of tubercle bacilli ("Gluckson" and "Bov. Vir.") died within thirty to fifty days so that observations beyond this were not possible, while those receiving the avirulent ("Human") and heat-killed bacilli lived well beyond the ninety-day period and were examined after this. Microscopic examination of the pus taken from the local site of injection into the guinea pig after fifty days reveals so few bacilli or is entirely negative, so that interpretation becomes difficult if not valueless,[5] and in the case where highly virulent bacilli were used the animals did not survive far beyond the thirty-day interval. Thus this experiment might be considered to have failed in attaining its full purpose, yet the results noted may be considered of some significance and will be given only in brief here. In all the guinea pigs that lived and from which pus was obtainable, there usually were numerous bacilli present in the stained smears up to the twentieth day. Although there was a marked variation in size of the individual bacilli seen, the range in variations and forms, regardless of whether dead bacilli or living bacilli had been injected, corresponded closely to that of the six weeks' old culture injected originally, there being a few long forms, but the predominating form was the intermediate shorter bacillary forms common to human tuberculous sputums. Many possessed metachromatic granules and some had polar bodies, but acid-fast coccoid or very short bacillary forms were not seen Likewise the long strings of individual metachromatic granules were absent, the granules being noted only in the fainter stained bacilli. Regardless of whether the bacilli were injected in viable or heat-killed form, twenty to thirty days after injection into the guinea pig the pus contained so few bacilli that they were only found with difficulty, if at all. An examination of a large number of specimens from many animals only leaves one with the impression that the forms seen are those, not of marked accelerating growth or of senescence or the beginning of the death period, but the adult form of the period of negative acceleration according to Henrici. Further studies on the tubercle bacillus and the allied acid-fast microorganisms in its class will be required to elucidate more fully the forms predominantly found in tuberculosis as it occurs in man and animals, as well as to determine the significance of the metachromatic granules and the polar bodies found in certain forms of the bacilli.

DISCUSSION

The tubercle bacillus, in common with a number of nonpathogenic microorganisms of like type, possesses the property of acid-fastness, and, except for differences in rate of growth and animal pathogenicity, there is very little to serve as differentiating features. Three varieties of the acid-fast microorganisms (the human, bovine, and avian tubercle bacilli) possess pathogenicity and morphologically they resemble each other closely, so closely that it is doubtful whether one can differentiate them with any degree of accuracy by microscopic morphologic examination. Biologically the different

species differ markedly, and it is by biologic methods that our present classifications and differentiations have been possible. Animal pathogenicity, optimum growth temperatures, rate of growth and reaction curves have served in this. There have been many attempts to alter the tubercle bacillus culturally and morphologically by utilizing various modified mediums, chemical agents and unnatural conditions, but this has led only to an apparent temporary transformation, with immediate reversion upon restoration to one of the standard mediums and conditions of growth. These temporary morphologic transformations (involution forms) become perfectly clear when one recognizes that the closely allied acid-fast organisms undergo certain progressive changes in growth which can be varied, as pointed out by Henrici, by any factors interfering with the normal growth curve or by the time at which the examination occurs. The acid-fast microorganisms included in this study, three rapidly growing acid-fast strains, an avian strain, two human strains and two bovine strains, revealed approximately the same morphologic changes—cytomorphosis—even though the rate of growth was markedly different for the individual strains. In the culture tube under suitable environment (temperature, culture medium, etc.) the bacillus can undergo morphologic transformation which when duly considered from the time standpoint give us the consecutive stages in its development. The acid-fast microorganisms, studied culturally in the light of the recent contributions by Henrici, make it evident that the young forms consist of uniformly-staining, long, straight and curved acid-fast filaments, the adult forms being shorter bacillary forms with metachromatic granules and the senescent form being uniformly stained acid-fast coccoid and very short bacillary forms. This is in entire accord with the conceptions of Wolbach and Ernst, who studied only human and bovine cultures of tubercle bacilli. A correlation of this with the animal studies makes it appear evident that the usual form seen in pus from experimental tuberculosis in animals (guinea pigs) is the adult form of tubercle bacilli; only occasionally is the young or embryonic form seen, and extremely rarely if at all the senescent form. It appears also that the adult form is the usual form encountered in tuberculous sputums from open cases of pulmonary tuberculosis.*

SUMMARY

Rapidly developing acid-fast nonpathogenic tubercle bacilli (Smegma, "Koch-Novy," and "Day") and rapidly and slowly developing pathogenic tubercle bacilli (human, bovine and avian), grown upon glycerol-agar and egg mediums, undergo a regular metamorphosis—cytomorphosis (Henrici)—which can be correlated with the time and growth curve of the bacilli. The period of early (accelerating) growth is identified with the presence of a large number of long filamentous, uniformly staining, acid-fast bacilli, while the period of adult (phase of negative acceleration) growth is identified with a preponderance of moderately long bacillary forms with and without metachromatic granules, and the senescent cells consist of uniformly acid-fast

*Note. For kind assistance in performing the technical details of this study the author is grateful to P. Cosman and Mary Moore.

coccoid and short bacillary forms. Correlated with macroscopic observations and cultural transplant studies, for slow growing human and bovine tubercle bacilli, the young embryonic forms were evident during the active period (first few months) of macroscopic growth on glycerol-agar and egg mediums,[12] while the senescent forms predominated (after eight to nine months), when subculturing to these mediums became more difficult or unsuccessful.

The tuberculous pus resulting from the subcutaneous injection of large amounts of living or heat-killed human or bovine tubercle bacilli (from a culture six weeks old) into guinea pigs, revealed predominantly the adult forms of tubercle bacilli. There was a gradual decrease in the number of bacilli present microscopically in the pus whether produced by living or heat-killed bacilli until that obtained after fifty days contained only a few bacilli or was negative. During the period of observation possible, there did not seem to be any change in relations of the forms of bacilli present in the pus at the local site of injection, and the virulence of the strain seemed to play no part, although the animals infected with the virulent strains died of a generalized tuberculosis within approximately one month after infection. Further and more exhaustive experiments are, however, necessary to be conclusive on this point and to elucidate this problem in so far as it concerns tuberculosis in man. It appears that the adult form of human tubercle bacilli[18] predominates in the sputum, as seen in routine examinations, from the majority of cases of pulmonary tuberculosis in man.

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EPINEPHRIN SHOWN CHEMICALLY IN THE BLOOD. DETERMINATION DIFFERENTIATING EPINEPHRIN FROM GLUCOSE IN THE BLOOD

(Preliminary Report)

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THE blood-sugar field is at present very well cultivated. Still, two very striking points present themselves: the clinician is not satisfied with the present interpretation of blood-sugar findings; and the literature testifies to the uncertainty of the whole subject. The object of this paper is to show by chemical means the presence of epinephrin in both normal and pathologic blood-sugar findings. An attempt will be made to demonstrate that in many cases of apparent "hyperglycemia" we are actually estimating epinephrin and not sugar.

ABSTRACTS FROM LITERATURE

Lundsgaard and Holboell,[1] in an investigation of eleven normal subjects showed that a part of the blood sugar exists in a form possessing a specific rotation weaker than in A and B glucose. This was manifest during both fasting and digestion. This form of glucose is similar to the substance obtained in vitro from A and B glucose under the effect of insulin or fresh muscle tissue.

In a second report[2] these investigators present evidence that the change in the specific rotation of light by a solution of A and B glucose after the addition of fresh muscle tissue and insulin is due to the production of a new form of glucose with a low specific rotating power. They explain it as the first phase of carbohydrate metabolism and call it the "new" glucose.

Winter and Smith[3] have recently advanced the suggestion that the sugar in normal blood is the highly reactive isomer, A-glucose, while in cases of severe diabetes the blood sugar is the relatively stable B form. They based this hypothesis upon observations that deproteinized extracts of normal blood when examined polariscopically over a period of three days show a progressively decreasing rotation. The power of these same extracts to decolorize potassium permanganate also decreases. Deproteinized bloods of severe diabetics, on the other hand, showed this phenomenon not at all or to a greatly lessened degree.

In another paper, Winter and Smith[4] present a note upon work done upon rabbits made hyperglycemic by means of epinephrin injections. In this paper they state that in this condition the mixture of sugars present in the blood would appear to be similar to that existing in diabetic blood before the injection of insulin. They advance the theory that the cause of diabetes is the absence or inactivation of an enzyme whose function it is to transform the relatively stable A B glucose into the exceedingly unstable A form.

Visscher[5] corroborated the findings of Winter and Smith with normal blood but found that the difference between normal and diabetic blood could be determined by varying the hydrogen-ion concentration of the deproteinized extract.

Denis and Hume,[6] upon repetition of the work, consider the claims of Winter and Smith unjustifiable and lacking in proof.

Hiller, Linder, and Van Slyke,[7] after subjecting blood to yeast fermentation and incubation at 38° C. under conditions that lead to complete destruction of glucose, conclude that there is a residue of a reducing substance in the blood other than glucose.

Physiologically, Tournade and Chabrol,[8] after having joined the suprarenal vein of one dog to the jugular vein of another, noted that centripetal irritation of the sciatic or crural nerve in the donor induced in the other dog a slight hyperepinephrinemia. The latter was manifested by contraction of the spleen, with or without changes in the arterial pressure.

In a later paper[9] they describe experiments upon dogs with *one* or both suprarenal glands *removed* and affirm that the amount of epinephrin poured out into the blood by a *normal* gland plays an unmistakable physiologic rôle in the organism.

Gley and Quinquad's[10] research upon cats showed that venous blood drawn *from* the suprarenal glands during irritation of the splanchnic nerve, manifested the properties of epinephrin when reinjected into the same animal.

The determination here described is based upon the fact that epinephrin reacts with picric acid and sodium carbonate with heat, producing a red color identical with that given by glucose. Since both glucose and adrenalin give this same red color, it is obvious that one must be removed. Iron is absolutely destructive to epinephrin and is, therefore, employed to meet this end. Two sugar determinations are run one with, the other without iron, the former being the true sugar content.

EXPERIMENTAL

Purpose.—To show that ferric chloride destroys epinephrin but has no effect upon pure glucose.

Procedure.—A solution of glucose was made at random, about 0.3 per cent strength from the c. p. crystals. A solution of adrenalin in 0.5 per cent hydrochloric acid was made from the c. p. crystals (Parke, Davis and Company). A few crystals from a one-grain vial were dropped into a test tube, quantity indefinite.

(Iron wherever used was filtered out in the following manner: A small 2.5 cm. funnel was employed. Flask contents were emptied into the filter, which were permitted to drain completely into a volumetric flask. They were washed with 1 or 2 c.c. of water which was added to the original flask, rotated well, and then poured into the funnel, washing the paper carefully. The funnel was allowed to drain completely before further washings were added from the original flask. About 4 or 5 washings were required, the funnel being emptied completely each time before the addition of further washings. The solution was cooled and then made up to volume. The last filtrates came through water-clear, and the paper was absolutely free from any color of the solution.)

1a. Exactly 1 c.c. of the glucose solution was pipetted into a 150 c.c. Erlenmeyer flask. Exactly 10 c.c. of saturated aqueous solution of picric acid were added, followed by 2 c.c. of 10 per cent sodium carbonate. Contents were boiled over a small, free flame almost to dryness; they were then transferred quantitatively to a 25 c.c. volumetric flask, cooled, and made up to mark.

1b. Same as above, except that a few small pieces of ferric chloride were added before the addition of the sodium carbonate. The final solution was heated and filtered.

2a. Exactly 3 c.c. of the adrenalin solution were pipetted into a similar flask and treated as in *1a.*

2b. Same as *2a,* except that ferric chloride was added before the addition of the sodium carbonate. The final solution was heated and then filtered.

3a. Exactly 1 c.c. of the sugar solution and 3 c.c. of the adrenalin solution were treated as in *1a.*

3b. Same as *3a,* except that ferric chloride was added. The final solution was then heated and filtered.

The solutions were compared with each other.

STANDARD	READINGS
1a. set at............10.00 mm.	*1b*..................10.00 mm.
2a. " "............10.00 mm.	*2b*..................11.00 mm.
1a. " "............10.00 mm.	*3a*.................. 9.00 mm.
1a. " "............10.00 mm.	*3b*..................10.00 mm.

Fearing that some of the adrenalin might have been destroyed in the acid solution, the above was repeated as follows:

The same glucose solution was used. Adrenalin crystals were dropped into a beaker containing 10 c.c. of saturated picric acid solution. Solution is instantaneous. Five c.c. of this solution were transferred to the Erlenmeyer flask, and 5 c.c. additional picric acid were added, making 10 c.c. in all. The remaining 5 c.c. were used in the twin test. Procedure the same as the previous. Comparisons:

STANDARD	READINGS
1a. set at............10.00 mm.	*1b*..................10.00 mm.
2a. " "............10.00 mm.	*2b*..................17.90 mm.
1a. " "............10.00 mm.	*3a*.................. 5.50 mm.
1a. " "............10.00 mm.	*3b*.................. 9.70 mm.
3a and *3b* were compared	
3a. set at............10.00 mm.	*3b*..................14.20 mm.

This shows conclusively that sugar is not affected.

WORK DONE ON HUMAN BLOOD

After having determined the glucose and the epinephrin content of various human bloods, glucose or adrenalin (Parke, Davis and Company), and in some cases both, were added and studied as indicated. In all this work no attention is paid to the quantity of either substance added, for the reason that glucose is known to react quantitatively, the object being to show: first, that added adrenalin intensifies the color of the sugar reaction; and, second, that adrenalin is destroyed by ferric chloride. See Table I.

TABLE I

NO.	TREATMENT OF BLOOD	READING	DILUTION	PRESENT FIGURE AS IS EPINEPHRIN AND GLUCOSE	READING	DILUTION	EPINEPHRIN DESTROYED—GLUCOSE ONLY
		mm.	c.c.	mg.	mm.	c.c.	mg.
1	Ppt. 15 minutes after bleeding	3.9	10	256.4	4.0	10	250.0
	Adrenalin added, part ppt., immediately	7.2	25	347.0	3.4	10	294.1
	Blood containing adrenalin left standing at room temperature for 24 hours	3.7	10	270.2			
2	Ppt. 15 minutes after bleeding	3.8	10	263.1	3.9	10	256.4
	Left standing as is room temperature for 24 hours	15.5	10	64.5			
	After standing adrenalin added, ppt., immediately	4.8	10	208.3	7.0	10	142.8
3	Ppt. 30 minutes after bleeding	7.1	10	140.8		Lost.	
	Glucose added to one portion, ppt., immediately	3.6	25	694.2	3.6	25	694.2
	Adrenalin added to other portion, ppt., immediately	1.4	25	1785.5	5.1	10	196.0
4	Ppt. one minute after bleeding	6.8	10	147.0	7.4	10	135.1
	Glucose and adrenalin added, part ppt., immediately	2.8	25	892.7	3.2	25	781.2
	Blood with added sugar and adrenalin left standing room temperature 24 hours	1.7	10	588.2			
5	Ppt. 5 minutes after bleeding	13.2	10	75.7	15.6	10	64.1
	Glucose and adrenalin added, ppt. at once	3.2	25	781.2	4.6	25	543.2
	Same left standing room temp., 24 hr.	4.1	25	609.7	4.1	25	609.7
	Same left standing room temp., 48 hr.	14.1	10	70.9	14.1	10	70.9
6	Ppt. 15 minutes after bleeding	7.3	10	136.9		Lost.	
	Glucose and adrenalin added, part ppt., immediately	4.1	10	243.9	5.5	10	181.8
	Left standing room temp., 24 hr.	4.3	10	232.5	4.5	10	222.2
	Same left standing room temp., 48 hr.	18.7	10	53.4			
7	Ppt. 30 minutes after bleeding (diabetes)	3.8	10	263.1	3.8	10	263.1
	Left standing room temp., one week; glucose and adrenalin added, ppt., immediately	1.5	10	666.6	2.3	10	434.7
	Same left standing room temp., 24 hr.	3.1	10	322.5	3.1	10	322.5
	Given insulin, bled one month later, as is	3.3	10	303.0	3.3	10	303.0
	Left standing room temp., 24 hr.	3.5	10	285.7			
	After standing, adrenalin and glucose added, ppt., immediately	3.3	50	1515.0	2.4	10	416.6
	Left standing room temp., 24 hr.	3.5	10	285.7	3.5	10	285.7
	Left standing room temp., 48 hr.	6.4	10	156.2			
	Left standing room temp., 60 hr.	6.4	10	156.2			
	Bled one month later, on insulin	4.7	10	212.7	5.3	10	188.6
8	Ppt. one minute after bleeding	11.5	10	86.9	20.0	10	50.0
	Glucose added, ppt., immediately	5.1	10	196.0	5.1	10	196.0
	Left standing room temp., 48 hr.	7.0	10	142.8			
	Adrenalin added, ppt., immediately	6.1	25	409.7	10.0	10	100.0
	Left standing room temp., 48 hr.	Color nil.					
9	Ppt. 10 minutes after bleeding	2.6	10	384.6	2.8	10	357.1
	Left standing as is room temp., 24 hr.	2.6	10	384.6			
	Left standing 48 hr., room temp.	2.6	10	384.6			
10	Plasma separated and left standing at room temperature one week						
	To part, glucose was added, ppt., immediately	1.3	50	3846.0	1.3	50	3846.0
	Left standing room temp., 24 hr.	1.6	50	3125.0			
	To other part adrenalin was added, ppt., immediately	5.0	10	200.0	8.2	10	121.9
11	Plasma separated, glucose and adrenalin added, ppt., immediately	2.7	10	370.3	4.1	10	243.9
	Left standing room temp., 24 hr.	2.7	10	370.3	4.1	10	243.9
	Left standing 48 hr.	5.8	10	172.4	8.4	10	119.0

TABLE II

	SUBJECT	BLOOD PRESSURE	BLOOD SUGARS: READING FIRST TEST	BLOOD SUGARS: PRESENT FIGURE AS IS EPINEPHRIN AND GLUCOSE	BLOOD SUGARS: READING SECOND TEST	BLOOD SUGARS: EPINEPHRIN DESTROYED—GLUCOSE ONLY
1	*Female adult*	mm.	mm.	mg.	mm.	mg.
	Patient had had supper at 7:00 P.M.					
	First blood drawn at 8:30 P.M.	117.7	11.3	88.4	15.6	64.1
	10 minims injected: 5 minutes, B. P., 135.0 mm.; began to lower					
	10 minims more injected: 2 minutes	132.0				
	Second blood		9.5	105.2	12.7	78.7
2	*Male adult* (Addison's disease)					
	Patient had had supper at 7:00 P.M.					
	First blood drawn at 10:00 P.M.	105.0	11.4	87.7	16.3	61.3
	30 minims injected: 3 minutes	160.0				
	Second blood		7.2	138.8	14.0	71.4
3	*Male adult*					
	Fasting stomach 9:00 A.M., 1" blood	105.0	9.1	109.7	9.1	109.7
	10 minims injected; 5 minutes	150.0				
	Second blood		6.6	151.5	8.4	119.0
4	*Male adult*					
	Fasting stomach 9:00 A.M., 1" blood	90.0	10.4	96.1	15.0	66.6
	20 minims injected: 5 minutes	164.0				
	Second blood		6.1	163.9	13.1	76.3
5	*Female adult*					
	Patient had had supper at 6:00 P.M.					
	First blood drawn at 8:30 P.M.	85.0	11.3	88.4	16.1	62.1
	20 minims injected; rise to 120.0 mm., immediate drop. 5 minutes, 20 minims more injected: 3 minutes	136.1				
	Second blood		8.0	125.0	13.9	71.9

A few patients received injections of adrenalin during their regular course of treatment. In this work adrenalin (1:1000 P. D.) was used. Injection was made into the buttocks. Blood was drawn from the median basilic vein. A Tycos sphygmomanometer was kept on the arm throughout the entire procedure. All of the blood sugars were precipitated immediately, necessary apparatus and reagents having been taken to the bedside when the patient was not ambulant.

From Table II it appears that hyperepinephrinemia and not hyperglycemia results from the injection of epinephrin. Increase of color density and rise of blood pressure are shown. The recovery of epinephrin from the blood of the patient is also demonstrated. In this connection, a recent work of Ohara[11] is of particular interest. In rabbits whose liver glycogen was made to disappear almost entirely through starvation, injections of epinephrin were noted to cause a very conspicuous *hyperglycemia.* Repeated injections of adrenalin developed a *glycosuria,* and marked *hyperglycemia.* Ohara also produced a marked *hyperglycemia* in hepatectomized rabbits. Inference is made by Ohara, that the

hyperglycemia produced in these hepatectomized animals might be due to the breakdown of the glycogen in the muscles. Ohara's work appears to confirm the results just given that hyperepinephrinemia and epinephrinuria are produced by injections of adrenalin.

METHOD

It is important that the blood be precipitated immediately, or as soon as possible, after bleeding, if the results are to be of any value.

Pipette 2 c.c. whole oxalated blood into a 25 c.c. volumetric flask. Add a saturated aqueous solution of picric acid and make up to mark. Shake well to insure thorough mixing, filter. Two 8 c.c. aliquots are required. Measure one 8 c.c. portion into a 150 c.c. pyrex Erlenmeyer flask. Add 2 c.c. of 10 per cent sodium carbonate solution and boil over a small, free flame until precipitation occurs. Dissolve in 1 or 2 c.c. of water, transfer quantitatively to a 10 c.c. volumetric flask, cool, and make up to volume. Pipette the other 8 c.c. aliquot into a similar flask, add about 50 or 80 mg. Fe_2Cl_6 (about 2 or 3 small pieces). Add 2 c.c. 10 per cent sodium carbonate solution and boil as before.

Add 1 or 2 c.c. water, filter through a small 2.5 cm. funnel into a 10 c.c. volumetric flask. Permit the funnel to empty completely; add 1 or 2 c.c. water to the original flask, and pour into the funnel, carefully washing the paper. Repeat until almost up to mark, allowing complete filtration each time before adding washings. Cool and make up to mark. This takes about 4 washings. When the operation is complete, the paper should be absolutely free of any color from the solution, and the filtrate should come through water-clear.

Comparisons should be made at once in any standard colorimeter. Standard blood-sugar solution* is used and is set at 10.00 mm.

$$\frac{\text{Reading of Standard}}{\text{Reading of Unknown}} \times 100 = \text{mg. per 100 c.c.}$$

Reading of first determination is sugar and epinephrin. Reading of second determination is sugar only (epinephrin has been destroyed).

Color equivalent for epinephrin has not as yet been determined. This test is simply to differentiate between the actual sugar content and that color which is due to epinephrin. Attention is here called to an observation throughout this work of a slight increase over the actual sugar figure after the added or injected epinephrin has been destroyed. No explanation is offered for this as yet. Following is a list of some of the unselected cases studied. All diagnoses were made by the clinician.

DISCUSSION

While glucose reacts with certain reagents which form the basis of blood analytic procedures, it is recognized that none of them is entirely specific for glucose. In various conditions, as for example, severe nephritis, hypertension (some forms), certain cardiac conditions and hyperthyroidism, there is an

*Permanent Standard

Picramic acid	0.064 gm.
Na_2CO_3 (anhydrous)	0.100 gm.
Distilled water to mark	1000.000 c.c.

Standardize against pure glucose.

TABLE III

CASE NO.	SEX	CONDITION	READING FIRST TEST	PRESENT FIGURE AS IS, EPINEPHRIN AND GLUCOSE	READING SECOND TEST	EPINEPHRIN DESTROYED—GLUCOSE ONLY
1492	M	Diabetes mellitus	3.9	256.4	4.0	250.0
1523	F	Diabetes mellitus	3.8	263.1	3.9	256.4
1249	M	Diabetes mellitus (insulin case)	3.7	270.0	5.3	188.6
1306	M	Diabetes mellitus (treated) nephritis	2.4	416.4	2.6	384.6
1384	F	Diabetes mellitus (treated) pyelitis	2.7	370.0	3.3	303.0
1786	F	Diabetes mellitus, essential hypertension	3.4	294.1	6.2	161.2
1326	F	Diabetes mellitus, 3/20/24	4.9	204.0	Lost	
		Same, 3/31/24	3.8	263.1	3.8	263.1
		Same, 4/16/24	2.4	416.6	2.4	416.6
		Same, 4/30/24, insulin started	3.3	303.0		
		Same, 5/13/24, gangrene, exitus	4.7	212.7	5.3	188.6
	M	Diabetes mellitus, after treatment	3.3	303.0	5.0	200.0
	F	Diabetes mellitus, B. P., 200 mm.	3.5	285.7	7.4	135.1
	M	Diabetes mellitus, neuritis (reading x 2)	6.5	309.2	6.5	309.2
[illegible]47	F	Diabetes mellitus (changed doctors)	2.6	384.6	2.8	357.1
3372	F	Diabetes mellitus (insulin)	4.2	238.0	7.8	128.2
1467	F	Toxic hyprtension, B. P. 200/130, obesity, nephritis, hyperthyroidism	3.0	333.3	3.5	285.7
1410	F	Essential hypertension, B. P., 195/100, arthritis	4.5	222.2	9.6	104.1
1336	M	Essential hypertension, mild pyelitis	7.1	140.8	9.3	107.5
1558	M	Hypertension, occasional glycosuria	7.1	140.8	Lost	
1431	F	Questionable diabetes, hypertension	6.5	153.8	7.0	142.8
2029	F	Nephritis, arthritis, essential hypertension	5.8	172.4	11.7	85.4
3228	M	Essential hypertension	5.2	192.3	16.0	62.5
1494	F	Polyarthritis, possible hyperthyroid	2.9	344.8	2.9	344.8
1318	F	Arthritis, nephritis, hyperthyroidism	4.8	208.3	4.8	208.3
1631	M	Obesity, alcoholism	5.4	185.1	6.0	166.6
3517	F	Arthritis, hypertension	2.5	400.0	4.6	217.3
1645	F	Arthritis, autointoxication	9.3	107.5	11.8	84.7
1614	F	Hernia	7.7	129.8	7.7	129.8
1764	M	Fatigue, no diagnosis to date	6.2	161.2	10.2	98.0
1618	F	No diagnosis	6.8	147.0	7.4	135.1
1672	M	No diagnosis	9.8	102.0	11.7	85.4
1754	M	Nephritis, hypotension	13.2	75.7	15.6	64.1
1864	F	No diagnosis	10.4	96.1	13.7	72.9
1872	M	No diagnosis	10.9	91.7	14.5	68.9
1778	F	Arthritis, hypotension	12.1	82.6	17.5	57.1
1869	F	Chr[illegible]n, nephritis, arthritis, infantile uterus	10.8	92.5	15.4	64.5
1882	M	Ast[illegible]igo, hypotension	10.9	91.7	10.9	91.7
1897	M	No [illegible]	7.8	128.2	14.7	68.0
1909	F	No [illegible] (hypotension)	12.1	82.6	15.2	65.2
1947	M	[illegible]ia, mild nephritis, hypotension	11.4	87.7	15.0	66.6
1964	M	Ad[illegible]se, possible Ca . . ?	10.7	93.4	11.5	86.9
		Sa[illegible]h's later	11.4	87.7	16.3	61.3
		Sa[illegible]ter, die[illegible]ddison's	21.9	45.6	21.9	45.6
[illegible]8	F	To[illegible]ion, pyo[illegible]onsils, teeth)	10.2	98.0	14.5	68.4
[illegible]	M	L[illegible]	9.6	104.1	15.5	64.5
[illegible]	F	Es[illegible]ension	7.2	138.8	13.8	72.4
[illegible]	F	S[illegible]	7.4	135.1	11.3	88.4
	M	[illegible]	10.8	92.5	10.8	92.5
	F	[illegible]	9.2	108.6	16.4	60.9
	F	N[illegible]	9.9	101.0	21.6	46.2
	F	F[illegible]	11.3	88.4	20.1	49.7
		[illegible]	9.6	104.1	28.0	35.7

TABLE III—CONT'D

CASE NO.	SEX	CONDITION	READING FIRST TEST	PRESENT FIGURE AS IS, EPINEPHRIN AND GLUCOSE	READING SECOND TEST	EPINEPHRIN DESTROYED—GLUCOSE ONLY
3318	M	Moderately increased blood pressure	8.3	120.4	14.2	70.4
	F	Hypertension	7.3	136.9	13.5	74.0
3399	M	No diagnosis	8.5	117.6	10 5	95.2
3469	M	No diagnosis	9.2	108.6	20.5	48.7
3505	M	Glycosuria	9.5	105.2	15.0	66.6
3478	M	Asthenia, chronic bronchitis, tonsils, hypotension, B. P. 90/50	9.3	107.5	13.8	72.4
3527	F	Arthritis?	2.5	400.0	4.6	217.3
3527	M	Asthenia, hypotension, toxemia, tonsils (following tonsillectomy, B. P. normal)	9.7	103.0	13.8	72.4
3541	M	Gastric ulcer	10.1	99.0	15.0	66.6
1662	M	B. P. 200/150	11.4	87.7	12.2	81.1
3661	F	Very obese, tonsils, possible growth?	9.8	102.0	15.0	66.6

hyperglycemia which has no explanation. Epinephrin, as shown in another paper,[12] reacts with picric acid and gives all the reduction tests identical with those of glucose. Since, therefore, as shown in this paper, it increases the color density of the blood sugar, and since it is known to cause a rise in blood pressure, it seems conclusive, in some instances at least, that we are estimating epinephrin and not glucose.

A discussion on diabetes is entirely too vast and out of the scope of this paper; still a few remarks will not be amiss. The pancreatic theory of diabetes has by far the greater number of followers and, with the discovery of insulin, the long surmised pancreatic internal secretion controlling carbohydrate metabolism has been confirmed; still the pancreas as the seat of structural alteration is questionable, for, according to Allen,[13] it is striking how much degeneration of the pancreas can be produced without diabetes, in contrast to the relatively slight changes found in diabetic men and animals. Epstein[14] states that while it is believed that the disease is due to a deficient production of insulin, the exact mechanism of the production of this condition is still problematic. This disease might, then, be the effect rather than the cause.

Returning to the subject of this communication, a prominent point relative to diabetes suggests itself. I do not want to be too emphatic in stressing this point, but from mere observation, it would appear that the epinephrin content of the blood stream in pancreatic diabetes is nil and on insulin treatment, with the decrease in the glucose content, there is a return of epinephrin to the normal and even to excessive quantity. Even in cases in which the blood sugar under insulin treatment cannot be decreased below a certain point, say 150-180 mg., the epinephrin content as per this test seems to rise, whereas the actual glucose content is greatly reduced, being normal in some cases and in others only slightly increased. No attempt is made to explain these phenomena. Can-

non, McIver and Bliss[15] and recently Müller and Petersen[16] have reported that the *injection of epinephrin* before *insulin retards* the marked decrease in the blood-sugar content. A coordination between the suprarenal glands and the pancreas might be suspected. I wish to emphasize again that the above is simply an observation based upon some 300 cases, which is entirely too small a number for conclusive deductions. It is simply included as a possible clue to the solution of the problem.

A change of the present normal blood-sugar figure is obvious. Benedict,[17] in discussing a new method, is of the opinion that the sugar content of blood averages about 75.0 mg. per 100 c.c., and that the actual glucose content of normal blood does not usually exceed 60.0 mg., except for a short period after the ingestion of food. From the data presented here, it would appear that the normal glucose content averages about from 55.0 or 60.0 mg. to about 75.0 or 80.0 mg. per 100 c.c., and that between 80.0 and 110.0 or about 20 mg. (terms of glucose) above the glucose content would be the normal epinephrin content.

Epinephrin is shown, contrary to accepted theory, to produce an hyperepinephrinemia and not hyperglycemia. This would then appear to confirm the supposition entertained by some that the cause of some forms of hypertension might be overactivity of the suprarenal glands.

SUMMARY

Epinephrin is shown to be a normal constituent of the blood stream. A determination is offered to differentiate between the epinephrin and the actual glucose content of the blood.

An attempt is made to demonstrate the difference between true hyperglycemia and hyperepinephrinemia; e. g., adrenalin added to human blood is shown to increase the color intensity of the blood-sugar reagent, which color is diminished almost to the color of the original sugar content after treatment with ferric chloride. (Pure glucose is shown to be unaffected by ferric chloride.) This is further shown by injections of adrenalin into human subjects. The blood pressure is shown to rise. There is an increase in the color density of the blood sugar and then upon addition of the Fe_2Cl_6 to the same filtrate the color density diminishes. Glucose does not react in this manner.

A new normal blood-sugar figure is suggested.

Such substances as uric acid, creatinine, creatine, glucuronic acid, lactose and purins, which might interefere (add to) with the blood-sugar determination were found to be *unaffected after treatment with ferric chloride.*

Attention is again called to the fact that the color equivalent of epinephrin has not as yet been worked out. Clinically, this will suffice for the present, for, if the *actual* glucose figure is determined, the excess color due to epinephrin, be it large or small, along with the clinical findings, is sufficient for diagnostic purposes.

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BASAL METABOLIC RATE IN SIMPLE AND PATHOLOGIC OBESITY*

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WHY will two individuals, each in the same apparent state of good health, show widely different tendencies to gain in weight?

The so-called normal individual seems immune to all sorts of dietary indiscretions. In spite of his habits of spending his energies as he likes and eating as much or as little as he pleases, he shows no more than a pound or two of change in weight over long periods of years.

Another individual, otherwise equally normal, must urge himself to vigorous exercise, and must continuously inflict upon himself dietary sacrifices, and in spite of these restrictions his weight mounts up and up as the years go by.

This is the problem of simple obesity. In discussing the problem of pathologic obesity, it will be necessary to digress from the main topic long enough to try to agree on what kind of cases shall be classed as pathologic.

Law of Conservation of Energy. Energy may be transformed from one form to another but may not be created or destroyed.

There are four forms of energy: (1) electrical, (2) mechanical, (3) radiant, and (4) chemical. When a given quantity of any one of these four forms of energy is transformed into another form, an equivalent quantity of energy reappears, because, although energy is transformable, it may not be created or destroyed. This principle of conservation of energy is fundamental to a consideration of the subject of obesity.

Animal tissues are capable of transforming only one form of energy, namely, chemical energy. This chemical energy arriving within the tissues as food is transformable into three forms of energy: namely, (1) radiant, (2) mechanical, and (3) chemical.

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The animal tissues are, like the food itself, stored *chemical* energy. If the chemical energy of the food circulating through the tissues be *in excess* of the body's immediate needs for radiant and mechanical energy, this excess must be "resynthesized" into chemical energy, and stored up along with the other tissues, because their excess cannot be destroyed and cannot escape from the tissues.

If the energy of the food supplied to the tissues be *insufficient* to meet the body's constant needs for radiant and mechanical energy, then, since this needed energy cannot be created, it must be supplied by transforming some of the chemical energy previously laid down in the body in the form of body tissues.

In other words, the entire mass of the body's tissues is the result of chemical energy in the form of food reaching the tissues *in excess* of the body's constant needs for radiant and mechanical energy.

Likewise, when chemical energy is supplied to the tissues in amounts *insufficient* to meet the body's needs for mechanical and radiant energy, there must result a transformation of the stored energy of the tissues to make up this shortage and, therefore, a loss in weight corresponding to the weight of the tissues so consumed for that purpose.

The body tissues and the foods are latent chemical energy, but they are rated as radiant energy, i.e., in terms of calories. For example, the latent chemical energy in fats, proteins, and carbohydrates of our food intake is transformable, respectively, into 9.3, 4.1, and 4.1 calories of radiant energy per gram when these are oxidized in the tissues, and these figures represent the so-called food values of fats, proteins, and carbohydrates.

A pound of dried muscle protein, therefore, contains 454 grams × 4.1 calories or 1860 calories. But muscle protein resynthesized into muscle tissue contains only 30 per cent of this 1860 calories, i.e., only 560 calories per pound of body weight, for the reason that muscle is 70 per cent water. Fat tissue on the other hand contains 4200 calories per pound of body weight, i.e., 9.3 × 454 grams = 4200 calories.

In other words, the growth of a pound weight of muscle in the body requires an excess of only 560 calories in the food intake, whereas, if stored as a pound of fat, an excess of 4200 calories is required.

Conversely, to lose a pound of fat which has been stored in the body as excess energy requires a shortage in diet of 4200 calories.

A person having a 2400 outgo and a 2400 intake per twenty-four hours would, therefore, be obliged to endure a shortage in the intake of food of 600 calories per day for an entire week in order to lose a pound of weight of fat.

1.—This radical reduction of 600 calories in the diet will induce almost constant hunger. 2.—The malaise or loss of both mental and physical stamina induced by this so-called "dieting" invariably causes a slowing up of the above-mentioned 2400 calories outgo, and thus partially or completely offsets the desired 600 calorie shortage. 3.—Restricting the diet below the normally required 2400 caloric intake causes a more economical absorption from the

intestines of food particles (especially fats, some of which would normally have been wasted as feces), thus again tending partially or completely to offset the desired 600 calorie shortage. 4.—After having endured constant hunger for an entire week or more, the individual unacquainted with the above mathematics of dieting, expects the scales to show a loss in weight commensurate with his dietary sacrifices and the hunger he has suffered. 5.—If the individual is then weighed as before on the same scales with the same clothing, the pound or more loss in fat tissues even then may chance to be offset by the weight of material in an unevacuated bowel or bladder. 6.—And finally, on a weight reducing diet, the fat consumed is often replaced by an equivalent weight of water, which is retained in the tissues and may amount to as much as eight pounds before any evidence of it begins to appear as edema. In such an instance one could live on a 600 calorie daily shortage for two months, and still find on weighing, no measurable reduction in body weight.

To any one unfamiliar with the law of conservation of energy and the mathematics of metabolism, such painfully prolonged demonstrations would be sufficient proof that diet has nothing to do with the body weight. This opinion, namely, that controlling the diet does not control the body weight, is prevalent, everywhere. If it were not, there would be no profit in the various nostrums, systems, drugs, and devices offered to the public for reducing excess body weight.

Even in the medical press, obesity has as many theories to explain it as there are remedies to cure it. The popular theory is that obesity is an hereditary characteristic, as seen in certain breeds of farm animals. Development of the normal bony structure, the circumference and length of the muscles under normal exercise, and also the sites chosen by the body for the deposition of adipose tissues are undoubtedly hereditary characteristics, but the *amount* of fat storage is not determined by heredity.

Another theory is that the specific dynamic action of protein is decidedly less marked in the obese than in normal individuals so that the obese can subsist indefinitely "on intakes much below their calculated caloric requirements."

Metabolic rate determinations on fourteen cases of simple obesity, using six normal subjects as controls, all of which were given a meal containing known amounts of protein, fats, and carbohydrates, showed practically the same rise in the metabolic rate proportionate to the amount of food ingested.

For example, a woman of 290 pounds weight and another woman of 110 pounds weight, after taking a 1,000 calorie dinner, showed, respectively, losses of 13 per cent and 12 per cent of this 1,000 calories calculated from the time of ingestion of the meal until the metabolic rates had dropped back to the basal rate, which was about six hours. Therefore, 13 per cent and 12 per cent of 1,000 calories = 130 and 120 calories, respectively, to be distributed over six hours of time, the period of absorption, or 21 and 20 calories respectively, per hour.

But this 21 calorie per hour increase in the obese subject's metabolism was only a 22 per cent increase over her 94 calorie basal metabolic rate, whereas, the 20 calories per hour increase in the normal subject's metabolism was a 37 per cent increase over her 54 calories basal metabolism.

From these figures it is seen that, although the obese subject utilizes the 1,000 calorie no more economically than does the normal subject so far as her response to the specific dynamic action of the food is concerned, the 22 per cent increase above her basal metabolism has the appearance of a decrease in rate as compared to that of the 37 per cent increase above the basal metabolism of the normal subject.

This difference in percentages is due solely to the fact that the percentage caloric loss, due to the specific dynamic action of the food, is compared in the one case with a large caloric output of a large body (94 calories) and in the other case, with a relatively small caloric output from a smaller body (54 calories). Thus her apparently diminished response to the specific dynamic action of food in the obese subject is due to the larger size of her body and not to her obesity.

Assuming that the specific dynamic action of food is less in the obese than in the normal person is simply another way of saying that if the obese person eats as much as the normal person he will be overfed, because of his more economical use of calories which the normal person wastes through a more vigorous response to the specific dynamic action of food. Then, if through this more economical use of his calories the obese person becomes *overfed, the same instincts which prompt the normal person to take* less food after he has been overfed, and thus avoid gain in weight, should also prompt the obese person to take less food and thus also avoid gain in weight. Therefore, the reduced specific dynamic action theory leads one no nearer the solution of the problem of obesity, because it does not explain why the obese person does not instinctively reduce his food intake when overfed as does the normal person.

According to another theory, obesity is practically synonymous with hypopituitarism, and a large percentage of fat persons, whether sick or well, are diagnosed as cases of hypopituitarism and treated with pituitary extract or with dangerous doses of thyroid extract.

How much the failure of development of the testicles influences the development and function of the pituitary, and how much faulty development of or malignant growth in or about the pituitary body influences the growth and function of the testicles, is mere speculation that has so far led to nothing of practical use in treatment.

Late appearance of the various centers of ossification, and delayed union of the epiphyses, as shown by the x-ray, is seen in both eunuchoidism and hypopituitarism. Tandler and Groz called attention to this years ago.

Outwardly, also, the eunuchoid and the hypopituitary patient look much alike in facial expression, retarded sexual development, and in the more or less characteristic distribution of adipose tissue.

These two conditions represent *two* types of the so-called Fröhlich's syndrome, or dystrophia adiposogenitalis.

The Fröhlich's syndrome of hypopituitarism has its seat of pathology in or about the pituitary, is usually malignant, becomes progressively worse, affects the general health, dwarfs the mental and physical development, and leads to obesity as the result of a reduced basal metabolism as well as a reduced general energy output caused by the stunted mental and physical life.

The Fröhlich's syndrome of eunuchoidism has its seat of pathology in the genitals, is not malignant, often progressively improves during adolescence, does not affect the general health, does not affect the mental or physical features except as related to the secondary sexual characteristics, and leads to obesity, not as a result of a reduced basal metabolism, but as a result of a reduced total energy output, in other words, to habitual laziness, characteristic of individuals who fail to develop the normal male sexual characteristics, as seen, for example, in castrated males of any species.

Obesity and the associated dysmenorrhea or amenorrhea are no basis whatever for making the wholesale diagnosis of hypopituitarism, so prevalent in certain sections of this country today.

One of our leading medical journals, in a recent editorial, questions the validity of the law of conservation of energy as applied to the problem of obesity. It flatly maintains that "the problem of gain in weight is not so simple as it is pictured." It says: "Experience tends to lead one to question whether the body always responds with such a nicety of regulation." This editorial also observes that "certain persons remain lean despite a liberal appetite and unimpaired digestion, whereas others deposit fat with apparent ease." And finally the editor asks: "Has not the time arrived for considering obesity as a scientific problem, and talking about it less glibly?"

According to this editorial and to widespread belief, two persons, one fat the other thin, may eat at the same table. The fat one on a restricted diet gains weight, while the thin one on a generous food intake remains thin. The fat one works hard all day, the thin one is continuously idle, but still the fat one grows more fat, and the thin one more thin.

Similar to this are the many arguments offered by those who disbelieve in the principle of balancing energy intake against energy outgo and who believe that their own personal observation of such cases is a better guide to truth than any theory.

The answer to all such argument is that even if it be not purposely misrepresented in any of its details, one is positively in no position to argue either side of the proposition until all of the calories of the food intake of both the fat and the thin person are known in *actual measured units,* and not referred to by such as the above inexact terms, "restricted diet" and "generous food intake."

Then the total calories of the energy outgo must be similarly actually measured and not referred to by such inexact terms as, "works hard all day," or, "is continuously idle."

cause in some cases the food intake has been decreased in proportion to the decrease in the energy outgo.

The cause of obesity seldom shows itself in the form of gluttony. Thus a normal 175 pound person at the age of twenty-five, taking at each meal-time a single mouthful of bread and butter in excess of the body's daily needs, will attain a weight of approximately 350 pounds by the time he is forty years old, and it is certain that no one could detect any evidence of gluttony when comparing two diets, one with and the other without the additional mouthful of bread and butter. Indeed it is of common observation that obesity is usually of extremely insidious onset.

The maintenance of a constant body weight under marked variation in bodily activity has also been the subject of frequent comment and wonderment.

The remarkableness of the phenomenon has been overrated. In a normal person, a gain in weight of five pounds of fat, when compared to the total body weight, it is true, is a small variation, but when compared to the three or four pounds of storage material already laid down, it is an enormous variation in gain of storage material. The accumulation of storage material is indeed about the least constant of the body's physiologic quantities. If wider variations in fat accumulations served a definite purpose in man's existence, as in the case of the hibernating bear, or the domestic hog, they would perhaps be in even greater evidence.

SUMMARY

1. The obesity of laziness and big eaters (simple obesity) and the obesity of eunuchoidism show no change in the basal metabolic rate, whereas the obesity of hypothyroidism, in both cretinism and myxedema, and the obesity of hypopituitarism, are associated with appreciable decreases in the basal metabolic rate.

2. Obesity is due to intake of food energy in excess of outgo of work and heat energy. Many factors influence the food intake. Similarly, many other factors influence the energy outgo. But no case of obesity can remain a mystery as to its cause if these factors have each been taken into consideration when balancing the total of the energy intake against the energy outgo.

3. In habitual undernutrition, the extreme opposite of obesity, the basal metabolism is markedly reduced. Reduction in the basal metabolism is, therefore, by no means necessarily associated with or followed by obesity.

4. The specific dynamic action of food, when calculated on the basis of the caloric intake, is not lower in the obese than in normals.

5. Many cases of obesity caused by a diet in excess of the normal body's needs (simple obesity) are often incorrectly diagnosed either as hypopituitarism or as hypothyroidism. Many cases of obesity due to eunuchoidism are also incorrectly diagnosed as cases of hypopituitarism.

6. Thyroid extract should never be used in the treatment of any form

of obesity in amounts sufficient to raise the basal metabolic rate to more than about 10 per cent plus.

7. Any attempt at reducing the body's energy intake of food, as in "dieting," is promptly compensated by the body's reduction in energy outgo, thus defeating the purpose of the dietary reduction, and too often effectively discouraging the patient with the "dieting" method of treatment for obesity.

SARCINAE IN PSORIASIS*

By Mary A. Marcus, A.B., M.S., Philadelphia, Pa.

REVIEWING the literature from an etiologic point of view, I was much impressed with the prevailing opinions as to the possibility of a parasitic nature of psoriasis.

The parasitic theory of psoriasis is strongly suggested from many points of view. Various organisms including fungi have been reported from time to time since 1853 when Wertheim[1] recovered a Penicillium from the urine of a psoriasis subject; later, 1879, Lang;[2] 1883, Eklund;[3] and, 1884, Wolff[4] also described mycelian fungi found in a lesion. These were later rejected by Neisser[5] and Reis[6] who showed that the alleged fungus was an artificial product of eleidin; in 1887 DeMattei[7] described a micrococcus, which he believed to be the causative agent, and produced psoriasis-like excrescences in inoculated animals, but this was regarded as an accidental finding by Majocchi.[7] In 1910 Sellei[8] described circular or ovoid, sharply outlined bodies found deep in the lesions in a case of psoriasis, and he regarded these bodies as the probable exciting cause. Crocker[5] also reported similar bodies, but their significance was not established. Pollitzer[9] and Haslund[10] believe that psoriasis is most probably due to an external microbic infectious agent; Mapather[11] is of the same opinion, but he adds that this microbic agent is an anaerobic one; Schamberg, Kolmer, Raiziss and Ringer,†[12] however, in their exhaustive study of the question of parasitism in psoriasis, arrived at no definite conclusion as to the parasitic cause of the disease. In this study they also reported an unidentified diplococcus, "X," isolated from five psoriatic lesions and from one blood culture; an ultramicroscopic, mobile, bacillar body demonstrated in seventeen out of nineteen psoriatics. Ultramicroscopic organisms resembling Paschen corpuscles of variola were reported by Sercowski and Wisnewski.[28]

More recently, 1919, Bory[13] also reports small, irregular, rod-like germs resembling mycelian threads which he found repeatedly in psoriasis lesions.

Many attempts to transmit the disease by direct inoculations have failed with the exception of two instances. In 1885, Lassar[14] was apparently able to show the transmissibility of psoriasis from man to rabbits. A rabbit was inoculated with blood lymph and psoriatic scales from a human subject, and with

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†These admirable studies should be consulted by all future investigators interested in the etiology of psoriasis.

similar material from this rabbit a second rabbit was inoculated and produced lesions suggestive of psoriasis. Behrend did not believe that these lesions conformed with the lesions in the human psoriasis, but according to his opinion they resembled tonsurans lesions.[21] Neisser,[5] Rindfleisch, Majocchi[7] and others failed to corroborate Lang. In 1887 Tenholt[15] ascertained that several individuals of a family contracted the disease from a dutch beef. The nurse in turn contracted the disease from these patients, but this malady could not be transmitted to the animal from the human subjects. Destot,[16] however, was apparently successfully inoculated from an infant who had vaccinal psoriasis; Ducrey,[17] DeAmicia,[5] Alibery,[5] and Hammer and Block[5] in a long series of inoculations, using men, rabbits, guinea pigs, and dogs, were unable to obtain any but negative results; Wutzdorff's and Schamberg's[18] efforts to inoculate themselves were also unsuccessful.

It is worth recalling that, in the above-mentioned studies made by Schamberg and his associates[12] in which antigen employed for syphilis was used, nine suggestive complement-fixation reactions, not accounted for by other evidences of syphilis, were obtained from 48 psoriatic subjects; and that some 16 different organisms, isolated from cases of psoriasis, were studied with the conclusion that there was lack of causal relation to the disease.

It seems to date that none of the foregoing findings have been either generally accepted or, in fact, sufficiently confirmed to lend even a strong suspicion for their specific pathogenicity of psoriasis.

The parasitic theory of psoriasis is further suggested by the fact that psoriasis follows vaccinations,[19] attacks of tonsillitis,[20] acute toxemias,[21] and various injuries, and by the fact that the border of one patch melts away when in contact with that of another.[22, 24] The disease also seems in some stages to be influenced favorably by exposure to the actinic rays of the sun. The distribution of the psoriatic lesions also suggests that the disease may be influenced by sunlight; Schamberg,[22] Knowles,[23] Stelwagon[24] and Ormsby[25] believe that the disease is much more prevalent in the winter months and in cold countries than in warm seasons and climates. Kayser[26] reported that in the tropics psoriasis is rare, and the symptoms of the disease, when they do develop, are rudimentary, the typical eruption scarcely ever being produced.

Sutton and others[27] believe that eradication of focal infection leads to prompter disappearance of the psoriatic eruption under the usual treatment.

In the bacteriologic study of the skin the technical difficulties are great. It has been noted from the literature that the predominating findings thus far obtained appear to be saprophytes, normally found in the skin. It is thus evident that in the study of a skin disease, such as psoriasis there are many complications incident to contamination by saprophytes; conclusions, therefore, are unreliable unless this contamination is reduced to a minimum. The following method was devised with this end in view.

METHOD OF STUDY

Materials were selected routinely from three different levels in such lesions, both for smears and cultures.

(1) The lesion was washed with 70 per cent and 95 per cent alcohol, and some of the scales were at once transferred to the culture media*; others were kept for an hour in 60 per cent alcohol and thereafter inoculated upon the culture media.

(2) After the scales had been removed, the second level was sterilized with 70 per cent and 95 per cent alcohol and irritated in order to provoke oozing of enough serum (lymph) to inoculate a culture media and for the preparation of impression smears and smears of mixed serum and scales.

(3) In making the third series of inoculations the exposed surface was again sterilized with alcohol, further irritated, and, from the renewed oozing, serum cultures and smears were prepared as before.

Three or not less than two lesions were thus treated; an average of ten inoculations and as many smears represented the extent to which the material was examined bacteriologically in each case.

(4) For taking blood cultures the surface of the forearm was sterilized as follows: The surface was washed with soap and water, alcohol and ether, and followed by mercurochrome 1:1000 in 50 per cent alcohol, and covered with dressing for a half hour.

The reason for the care in sterilizing the tissues prior to taking materials for cultures is that thereby one reduces the liability for surface contamination, if present, to intermix with the deeper causative agents.

(5) All smear preparations, made both from the cultures and directly from the lesions, were fixed (after drying in the air) by flame and in a 50:50 mixture of ether and alcohol. They were stained by Gram's, Muir's, Rosenow's capsule, and Giemsa's methods, respectively. Giemsa stain has proved equally good either in applying the stain directly on the smear for fifteen to fifty minutes or in staining them overnight and then washing them with the different solutions of acetone and xylol, as is the procedure for the tissue specimens. Muir's capsule method has proved very satisfactory for demonstrating the capsule of these sarcinae. (The carbolfuchsin was applied cold for an hour.)

RESULTS

Materials for this bacteriologic study were obtained from forty-one psoriatic subjects and from sixteen patients of other dermatoses.

In the examination of the lesions and blood in the forty psoriatic patients, thirty-eight (95 per cent) showed apparently identical sarcinae, and the remaining two (5 per cent) were negative. Five of the forty also showed staphylococci (12.5 per cent); three diplococci (7.5 per cent); two B. subtilus (5 per cent), and two B. pseudodiphtheria (5 per cent).

The same sarcinae (microscopically) have been demonstrated, so far, in fifteen smears made of the oozing serum and scales from the lesion, in eight smears of the scales and serum, in six of scales only, incubated (upon the same culture media given above) for seventy-two to ninety-six hours at 37° C., and in an impression smear made of the serum. Fifteen different psoriatic patients were thus examined.

*The culture media employed consisted of plain agar, glucose agar, maltose agar, Sabouround's and all other ordinary media.

Upon examination of the sixteen different controls, selected from other skin diseases, including eight cases of tinea circinata, three of pityriasis rosea, two of lichen planus, one of Duhring's disease, one of secondary syphilis and one of pellagra, a sarcinae was encountered in but one, that of pellagra. This organism lost its morphology in subcultures and reduced none of the sugars, which were affected by the sarcinae recovered from psoriasis. These atypical sarcinae were, therefore, excluded from further comparative study, especially since the usual method of the skin disinfection had not been followed. Ten additional cultures from normal skin areas of psoriatic subjects showed no sarcinae.

Blood cultures were made from three psoriatic patients, and from one of these (an acute case of two weeks' duration), similar sarcinae were obtained, but none from the two others (one three years' and the other of twenty years' duration).

Histologic sections made of the lesions of five psoratic subjects have thus far been carefully examined. Various staining methods were used which will be described in detail in the next paper. In two sections from two different subjects, stained by Muir's method, the sarcinae were successfully demonstrated. In each case the sarcinae were found in the epiderm, once near the scaling surface, the other time fairly deep in the epithelial layers and in the exudate.

In order to determine whether or not the sarcinae obtained from the psoriatic subjects were of the same species, further cultural and microscopic studies were made as follows:

CULTURAL CHARACTERISTICS

The primary growths of the sarcinae appeared to be invariably either large (3 by 5 mm.) elevated plate-like strata or very inconspicuous colonies on the media thus far employed. Often they were concealed in some of the blood clot transferred to the surface of the media and were apt to be overlooked and lost. The size of a single colony rarely exceeded 3 to 5 mm. in diameter. In the older examples and in the subcultures upon the agar media were usually very fine, raised, pearly, glistening colonies of dry, thick, plate-like growths of grayish-yellow or milky-gray color. Broth transplants were usually clouded with an ultimate production of a gray or grayish-yellow pellicle or sediment. In milk, an acid reaction usually occurred accompanied by coagulation (in only three instances the milk was rendered alkaline and no coagulation followed). Subcultures on potato were often invisible grossly, but again on the same raw potato there appeared heavy grayish-yellow or copper-orange discrete colonies. The Difco potato almost without exception gave rise to fine, round, raised, grayish-yellow to orange colonies.

Most of the thirty-eight cultures from psoriatic lesions produced acid in the ordinary sugar media, including dextrose, lactose, maltose, saccharose, levulose, mannite, dextrin, Russell double sugar and inulin. They reduced nitrates to nitrites, and occasionally liquefied gelatine. The biologic property of reducing sugars was, however, usually lost by old cultures and the growth was markedly retarded. It was observed that light inhibited growth and its absence hastened the growth, but with differences in the development. In one

	Bergey, Table I	Marcus, Table II
1.	Division occurs under favorable conditions in three planes.	Same division.
2.	Producing regular packets.	Producing regular and irregular packets of 2, 4, 8, 16, 32 and larger groups
3.	Growth on agar abundant, usually yellow, orange pigment.	Pigment usually whitish-gray or grayish-yellow.
4.	Dextrose broth slightly acid, lactose generally neutral.	Dextrose, lactose, maltose, saccharose, dextrin, mannite, inulin and Russell double sugar markedly acid.
5.	Gelatin frequently liquified.	Gelatin rarely liquified.
6.	Nitrates may or may not be reduced to nitrites.	Nitrates are usually reduced to nitrites
7.	No capsule reported.	Capsule demonstrated.

Table III

Sugar Reactions Produced by the Sarcinae Recovered from Psoriasis

No. of Patients	No. of Litmus Agar				Carbohydrates				Neutral Red Bouillon			
	Dextrose	Lactose	Maltose	Saccharose	Russell Double Sugar	Mannite	Dextrin	Triple S.	Inulin	Dextrose	Lactose	Maltose
1												
2	A	A	A	A	A	A	A	A	A	A	A	A
3												
4	O	O	A	A	ALK	O	O	?	A	A	A	A
5	A	A	A	A		O	O	O	?	A	A	A
6	O	?	A	?	ALK	O	O	?	?	A	A	A
7												
8	A	A	A	A	O	O	?	?		A	A	A
9	A	A	A	A	A	A	A	A	A	A	A	A
10	A	A	A	A	A	A	A	A		A	A	A
11	A	A	A	A	A				A	A	A	A
12	A	A	A	A	A	A	A	A		A	A	A
13	O	A	A	A	O	?	?	A	A	A	A	?
14	A	A	A	A	A	A	A	A	A	A	A	A
15												
16	A	A	A	A	A	A	A	A	A	A	A	A
17	A	A	A	A	A	A	A	A		A	A	A
18	A	A	A	A	A	A	A	A		A	A	A
19	O	A	O	O	O	O	O	A	O	A	A	A
20	O	O		A	O	O	A	O	O	A	A	A
21												
22	A	A	A	A	A	A	A	A	A	A	A	A
23												
24	O	O	A	A	ALK	O	O	?	A	A	A	A
25	A	A	A	A		O	O	O	?	A	A	A
26	O	?	A	?	ALK	O	O	?	?	A	A	A
27												
28	A	A	A	A	O	O	?	?	A	A	A	A
29	A	A	A	A	A	A	A	A	A	A	A	A
30	A	A	A	A	A	A	A	A	A	A	A	A
31	A	A	A	A	A	A	A	A	A	A	A	A
32	A	A	A	A	A	A	A	A	A	A	A	A
33	O	A	A	A	O	?	?	A	A	A	A	?
34	A	A	A	A	A	A	A	A	A	A	A	A
35												
36	A	A	A	A	A	A	A	A	A	A	A	A
37	A	A	A	A	A	A	A	A	A	A	A	A
38	A	A	A	A	A	A	A	A	A	A	A	A
39	O	A	O	O	O	O	O	A	O	A	A	A
40	O	O	A	A	O	O	A	O	O	A	A	A

A—acid; Alk—alkaline; O—neutral.

TABLE IV

SOME SPECIAL BIOLOGIC FEATURES OF THE SARCINAE RECOVERED FROM PSORIASIS

	666				LITMUS MILK						NITRATES REDUCED					THERMAL DEATH POINT							
					DAYS							NITRITES DAYS				WITH N/10 VOLERIC ACID			HEAT ONLY				
					1	2	4	6	8	10		2	4	6	8								
Patient's No.	Yellowish-Gray	Milky-Gray	Citro-Yellow	Milk-Coagul.							Indol Product.					Without Acid min.	Degrees of Temperature	Time—Minutes with N/10 Acid	Opt. Temp.	Capsule	Motility	Gelat. Liquef.	Gram's Stain.
1		+		+	A	A	A	A	A		+	+	+	+		20	80° C.	10	37°-40° C.		–	±	+
2	+	+		–	A	A	A	A	A		+	+	+	+		"	"	"	37°-40° C.	+	–	+	+
3		+		+	A	A	A	A	A		–	–	–	–		"	"	"	" " "	+	–	–	+
4		+		+	O	O	A	A	A		–	–	–	–		"	"	"	" " "	+	–	–	+
5		+		–	A	A	A	A	A		–	+	+	+		"	"	"	35°-40° C.	+	–	+	+
6	+	+		+	O	O	O	O	O		–	–	–	–		"	"	"	35°-37° C.	+	–	–	+
7	–	+														"	"	"	30°-35° C.	+		–	–
8	+	+		–	A	A	A	A	A		+	+	+	+		"	"	"	35°-38° C.	+	–	+	+
9	+	+		–	A	A	A	ALK	ALK		–	–	–	–		"	"	"	37°-40° C.	+	–	–	+
10	+		+	+	A	A	A	A	A		–	+	+	+		"	"	"	25°-35° C.	+	+	+	+
11	+				O	O	O	O	O			O	O	O		"	"	"	25°-35° C.	+	–	–	–
12	–																						
13	+	+		+	A	A	A	A	A		+	+	+	+		"	"	"	35°-38° C.	+	–	+	+
14	+		–	+	A	A	A	A	A		–	–	–	–		"	"	"	" " "	+	–	+	+
15	+	+		+	A	A	A	ALK	ALK		–	–	–	–		"	"	"	" 40° C.	+	–	–	+
16	+			–	A	A	A	A	A		+	–	+	+		"	"	"	" " "	+	–	+	+
17	+	+		–	A	A	A	A	A		–	–	–	–		"	"	"	" " "	+	–	–	+
18	+	+		–	A	A	A	A	A		–	–	–	–		"	"	"	" " "	–	+	–	+
19	+	+			O	O	O	O	O		–	–	–	–		"	"	"	" " "	–	–	–	–
20	–			–	O	O	O	O	O		–	–	–	–		"	"	"	37°-40° C.	+	+	+	+
21	–	+		–	O	O	O	O	O		+	–	–	+		"	"	"	" " "	+	+	–	+
22	+	+		+	ALK	ALK	ALK	A	A		+	–	–	+		"	"	"	" " "	–	–	+	+
23	–			–	A	A	A	A	A		–	–	–	–		"	"	"	" " "	+	–	+	+
24	–	+		+	A	A	A	A	A		–	–	–	–		"	"	"	" " "	–	+	–	+

TABLE IV—CONT'D

	666				LITMUS MILK DAYS						NITRATES REDUCED	NITRITES DAYS				THERMAL DEATH POINT WITH N/10 VOLERIC ACID			HEAT ONLY				
Patient's No.	Yellowish-Gray	Milky-Gray	Citro-Yellow	Milk-Coagul.	1	2	4	6	8	10	Indol Product.	2	4	6	8	Without Acid min.	Degrees of Temperature	Time—Minutes with N/10 Acid	Opt. Temp.	Capsule	Motility	Gelat. Liquef.	Gram's Stain.
25	−	+		−	A	A	A	A	A		−	−	−	+		20	80° C.	10	37°-40° C.	+	−	−	+
26	+	+		−	A	A	A	A	A		−	−	−	−		"	"	"	" " "	+	−	−	−
27																				−			−
28	+	+		−	A	A	A	A	A		+	+	+	+		"	"	"	" " "	+	−	−	+
29	−	+		−	O	O	ALK	ALK	ALK		−	−	−	+		"	"	"	" " "	−	−	+	−
30	+		+	+	A	A	A	A	A		−	−	−	−		"	"	"	" " "	+	−	−	+
31	−	+	−	−	A	A	A	A	A		+	−	−	−		"	"	"	" " "	+	−	−	+
32																				−	−	−	−
33	+		+	−	O	O	O	O	O		−	−	−	−		"	"	"	" " "	+	−	+	+
34		+		+	A	A	A	A	A		−	−	−	−		"	"	"	" " "	−	−	−	−
35		+		−	A	A	A	A	A		+	+	+	+		"	"	"	" " "	+	−	−	+
36	+			?	?	?	?	ALK	ALK		−	−	−	−		"	"	"	" " "	+	−	−	+
37	+	+		−	A	A	A	A	A		−	−	−	−		"	"	"	" " "	+	−	−	+
38		+	−	−	O	ALK	ALK	A	A		+	+	+	+		"	"	"	" " "	+	−	−	+
39		+		−	O	O	A	A	A		+	−	−	−		"	"	"	" " "	−	−	+	+
40	−	+		−	O	O	O	O	O		−	−	−	−		"	"	"	" " "	−	−	−	−
41																							
Total	20+	28+	3+	12+	23A 1ALK	23A 2ALK	24A 3ALK	25A 4ALK	25A 4ALK		12+	9+ −	10+	14+		38 in 20 min.	38 at 80° C.	38 in 10 min.	27 at 37°-40° C. 3 at 35-38 6 35-40	28+	4+ 6	14+ −	30+ −

Summary of Tables Nos. III and IV

Initial growth with thick elevated strata in cultures—1, 2, 3, 4, 5, 6, 9, 13, 19, 20, 22, 22	(12)
Initial growth with plate-like strata in cultures—1, 3, 5, 6, 23	(5)
Initial growth with elev. large moist opaque col. cultures—9, 15, 18, 22	(4)
Initial growth with med. dry glistening col. cultures—9, 10, 14, 22, 23, 24, 25, 26	(8)
Initial growth with fine pin-point dry col. cultures—9, 10, 21, 23	(4)
Subcultures with elev. large dry porcelain-white col. on maltose agar—2, 5, 22, 23, 24	(5)
Subcultures with elev. large moist yellowish-gray col. on maltose agar—9, 10, 15, 18	(4)
Subcultures with elev. large thick moist yellowish-gray surface growth on all agar sugars—9, 10, 15, 18	(4)
Subcultures with very fine granular almost invisible surface growth on all culture media including all other strains—	(33)
Subcultures with acid production on glucose—	(20)
Subcultures with acid production on lactose—	(26)
Subcultures with acid production on maltose—	(28)
Subcultures with acid production on maltose neutral red—	(30)
Subcultures with acid production on levulose—	(17)
Subcultures with acid production on saccharose—	(33)
Subcultures with acid production on Russell double sugar—	(15)
Subcultures with alkal. production on Russell double sugar—	(6)
Subcultures with acid production on mannite—	(16)
Subcultures with acid production on dextrin—	(19)
Subcultures with acid production on dextrose neutral red—	(39)
Subcultures with acid production on triple sugar—	(21)
Subcultures with acid production on inulin—	(17)
Subcultures with acid production on litmus milk—	(24)
Subcultures with alkal. production on litmus milk following acid reaction—	(2)
Subcultures with alkal. production on litmus milk preceding acid reaction—	(4)
Subcultures with reduction of nitrates to nitrites—	(14)

instance a culture which had appeared dead for about eight months, spontaneously began to show a secondary growth superficial to the old one, after it had been stored away from the direct sunlight for three months. This secondary growth appeared heavier, creamier, and more active than the ordinary culture, which was still inert.

It is therefore probable that dissociation of growth and the other biologic properties of the sarcinae are caused by both age and light.

MICROSCOPIC APPEARANCE

Microscopically the sarcinae, both in the initial and in as many as eight generations of subcultures, appeared as spheres averaging from 1.5 to 3.5 microns, arranged as pairs or in packets of 4, 8, 16, or even in groups larger than 32 or 64. Although the individuals in such packets were usually all equal in size, packets of eight spheres with one or more of the constituent spheres notably larger or smaller than the rest, thus deforming the usually regular cubes, were not infrequently found. When cubes stained by Gram's method were stained by Muir's method, either the cube or the individual coccus showed a distinct capsule.

The sarcinae are believed to be identical in all thirty-eight cultures, showing similar cultural and microscopic characteristics, but possessing distinct variations, amounting almost to pleomorphism,—a feature not uncommon in other known species of sarcinae. That these variations in the size of the individual spheres and packets, in pigmentation, in behavior in milk, in sugar media and liquefaction in gelatine are not specific differences is shown by the fact that such variations occurred occasionally with organisms derived from some

single colony, and that reversal of the variations were at times obtained. The details are given in Tables Nos. III, IV, and V.

HUMAN INOCULATIONS

Normal skin areas of twelve psoriatic subjects and one normal human volunteer were scarified and inoculated with pure cultures of the sarcinae isolated from psoriatic lesions; as a control a sterile 0.85 per cent salt solution was used. The inoculations were made about one inch apart. In a control of normal saline an erythema developed. At the site of the sarcinae injections there developed six out of twelve positive reactions. Three reactions following the sarcinae inoculation showed not only erythema and infiltration, but also a fine branny scaling and three weeks later, a white ring (suggesting that the tissue had been damaged and was not yet healed) around the infiltrated reddish center. One showed a very pronounced course with silvery scaling; in the other two only an erythema similar to that in the control appeared. Whether or not this is a specific action of the parasitic sarcinae or simply the result of mechanical irritation, as noted in certain cases by Schamberg, cannot yet be determined. The six psoriatic subjects, in whom the sarcinae inoculations were productive of reactions, included two subacute cases of about one year's duration each, two acute cases of two weeks' and two months' duration and two chronic cases with a history of three and twenty years, respectively.

ANIMAL INOCULATIONS

Experiments were conducted on one monkey and two rabbits; the monkey received five subcutaneous injections of 1 c.c. each of sarcinae suspensions, together with five scarification inoculations simultaneously on ten different areas of the skin; none of these as yet show any reaction. The same monkey and two rabbits received subcutaneously 0.5 c.c. sarcinae suspension into the outer side of the ear. A week later the areas inoculated of both species of animals were swollen and hard, and on excision showed a purulent discharge, from which by smear and culture the same type of sarcinae were demonstrated. No evidence of scaling, similar to that observed in psoriasis, had appeared during the one week interval prior to excision.

AGGLUTINATION AND COMPLEMENT FIXATION

If antibodies or agglutinins existed in the body fluids of psoriatic subjects in demonstrable amount, and if the antigen, presumably a parasite, were the sarcinae, this should yield with the antibody, complement fixation and agglutination, respectively.

SEROLOGIC TECHNIC

At the suggestions of Dr. J. A. Kolmer, several experiments were performed as follows:

Six different cultures of sarcinae isolated from the lesions of six psoriatic subjects were subcultured on agar, six times in succession and washed off with 0.3 per cent phenolated salt solution. The washings were shaken automatically for four hours and then heated to 60° C., for one and a half hours. Two mix-

tures of three pure sarcinae cultures each were prepared in a similar manner. Agglutination tests were performed with six of these antigens diluted 1:10, 1:20, and 1:40, respectively, against five psoriatic sera, one syphilitic serum, and one normal control serum; none showed agglutination.

Four of the same antigens and four psoriatic sera were employed for the complement-fixation tests* according to Kolmer's[31] new method. The results were all negative. Further research in serology is therefore projected.

DISCUSSION

In comparing the sarcinae isolated from the psoriatic lesion and the one blood culture, with Bergey's tabulation of the group of sarcinae, it is evident that the organism recovered belongs to Genus VII sarcinae (Goodsir).[29] Winslow and Rogers described it as follows: "Saprophytes and facultative parasites. Division occurs under favorable conditions in three planes, producing regular packets. Usually gram-positive. Growth on agar abundant, usually with formation of yellow or orange pigment. Dextrose broth slightly acid, lactose broth generally neutral. Gelatin frequently liquefied. Nitrates may or may not be reduced."

The essential differences of the sarcinae classified by Bergey[30] and the sarcinae isolated from psoriasis are shown in (Bergey's and in the writer's) Tables I and II.

It is a well-known fact that by the application of the actinic rays (when the psoriatic eruption is quiescent) the subject is often relieved of the psoriatic lesions; that the disease is influenced by sunlight is noted from the fact that the eruption usually occurs on the parts of the body covered by clothing, such as the elbows, knees, thorax, abdomen, and sacrolumbar regions. And further, the disease is worse in the winter and in cold climates, being better, disappearing or rarely occurring in the summer and in warm climates. It seems coincidental that the growth of the sarcinae, isolated from the psoriatic subjects, is inhibited by sunlight and thereby its biologic properties to reduce sugars are lost. (In the dark, at room temperature, the growth was observed to be active, but it was inhibited at room temperature in the presence of diffuse daylight, and, when the cultures were kept thus for ten weeks in the diffuse daylight, they would give no growth in repeated subcultures.) Active growth recurred after the old culture had been stored away from direct sunlight for three months and after eight subcultures had been made. A new growth of thick, creamy, heavy masses, superficial to the old dead surface growth occurred spontaneously, the old cultures being dead for about eight months.

A word may be said in favor of the technic devised for this study. In the forty-one subjects of psoriasis from which fifty cultures of bacteria have been obtained, only five showed staphylococcus, which is known to be the most common inhabitant of the skin, and only 5 per cent of diphtheroids, which is found in about 15 per cent of all skin cases.

*I am indebted to Miss Carola Richter of the Research Institute of Cutaneous Medicine, Philadelphia, for her kind assistance in the complement-fixation work.

Diphtheroids found in two blood cultures were reported by Schamberg, Kolmer and their associates. They also reported five other species of bacilli and ten different species of cocci, recovered from the lesions, which included a diplococcus "X" isolated from five lesions and one blood culture. All of these sixteen different species were based on the study of twenty-four psoriatic cases from which fifty-seven cultures have been obtained, but no sarcinae were reported.

In the present study of the forty psoriatic cases from which fifty cultures have been obtained, only five different species of bacteria were isolated, which included thirty-eight cultures (95 per cent) of one species of sarcinae isolated from the lesions and one blood culture, five cultures (12.5 per cent) of staphylococci, only about 7.5 per cent of diplococci, and but 5 per cent, two cultures each of B. subtilis and B. pseudodiphtheria isolated from the lesion only. *In some instances sarcinae were demonstrated* in the direct smear, while the cultures of the same case were negative. It would suggest that the organism was killed either by sterilization of the skin with alcohol or by treatment.

Thus it is readily seen that the technic used in this study largely obviates contamination.

In every case the cultures confirmed the findings of sarcinae in the fifteen direct smears, so far examined. Eight of the fifteen were made from the scales and oozing serum of the lesions, one impression smear made only of the serum (*by pressing the slide upon the irritated surface of the lesion*); six smears were made of scale and incubated at 37° C. for seventy-two to ninety-six hours. Sarcinae were also found in two histologic sections and in the one blood culture. It is worth recalling that the two sections showed the sarcinae not only in the epiderm but also once deep in the epithelial layers as well as in the cellular exudate.

Although no typical sarcinae were found in the fifteen control cases, selected from other skin diseases, and none were obtained in the additional control cultures made from normal skin areas of ten *psoriatic subjects, our* studies are being continued.

Even though six out of twelve sarcinae inoculations, by scarification in psoriatic patients, were productive of psoriatic lesions, it is unwarranted at the present time to assert the parasitic nature of the sarcinae to psoriasis, as one of the controls gave a similar reaction to that observed in the two last sarcinae inoculations. Therefore, whether the causative agent of the disease in question has or has not been isolated, only time and further study can tell. It is interesting to note, however, that the sarcinae here described have been isolated from the lesions studied in a proportion as high as 95 per cent.

Further attempts at serologic study, vaccine treatment and inoculations to reproduce the disease by different methods are being made. Especially the study to relate the histologic changes of the lesions with demonstrable sarcinae in all the psoriatic subjects studied bacteriologically must be completed in order to arrive at a definite conclusion as to the significance of the sarcinae found.

SUMMARY

1. A survey of the literature on the parasitology of psoriasis showed that most of the data thus far recorded appeared to be by the commoner saprophytes of the skin.

2. In order to overcome this contamination, a special method was devised.

3. The efficiency of this technic is shown by the fact that in 50 cultures from 40 psoriatics there were only 5 cultures (12.5 per cent) of staphylococci, 2 cultures each (5 per cent) of B. subtilis and B. pseudodiphtheria, and 3 cultures (7.5 per cent) of diplococci.

4. From the 50 cultures of 40 psoriatics, 38 strains (95 per cent) of a sarcinae apparently of identical species were obtained.

5. Sarcinae of the same morphology were demonstrated in 15 direct smears (15 patients) and also in histologic sections from two different patients.

6. Similar organisms were not recovered in cultures and smears of 16 controls (sundry scaly dermatoses), nor from the sound skin of 10 psoriatic subjects.

7. Six agglutination and four complement-fixation tests gave negative results.

8. Attempts to reproduce psoriatic lesions by inoculations into man and animals are in course and will be reported in detail in a later communication.

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DISCUSSION

Dr. Francis L. Burnett.—I have been interested in Miss Marcus' paper, because I have also been making a study of psoriasis. This disorder of the skin is supposed to be either inflammatory or metabolic in origin. In consideration of the infectious theory about which you have already heard, I wish to say a few words for the metabolic hypothesis. In the studies I have made in nutrition I have considered food factors from the absorption of digested products as determined by the form of the feces and the intestinal rate. Complete and proper nourishment from this point of view is a delicate and complex process in which many food factors have to be adjusted and generally controlled. Many of the patients with psoriasis eat too fast and too much or badly proportioned food; and on this account there is malabsorption. The skin fails to get the digested products essential for normal maintenance. By dietary treatment, when the patient is educated to eat slowly, select properly proportioned food, etc., there is a change in the intestinal rate and the anabolic processes are improved. Under these circumstances the lesions of the skin gradually disappear; and, if the patient continues treatment and the elimination of the faulty food factors, another attack will be prevented. Accordingly, from the metabolic point of view we not only have a very logical basis for the treatment of the disease, but also a means of maintaining the health of the skin.

Dr. Martha A. Wood.—What the previous speaker has said corresponds with my experience. In one case a patient came for examination of feces. I found that the feces were loaded with trichomonas and a too frequent rate of stools during the day. He was put under treatment for the intestinal condition and was cured.

Dr. John A. Kolmer.—Owing to the wide prevalence of psoriasis and the fact that in some cases the clinical history suggests a possible parasitic origin, the work reported by Miss Marcus properly engages our attention. As correctly stated by her it is doubtful whether the disease has ever been certainly transmitted to a normal human being or lower animal, although it is a common clinical experience that new lesions may be produced in the psoriatic cutaneous irritation. The apparent impossibilty of transmitting the disease to the lower animals has naturally greatly added to difficulties in etiologic studies in this disease; in a general way it also detracts from its parasitic origin.

At the Research Institute for Cutaneous Diseases where Miss Marcus has done a part of her work, both Dr. Schamberg and I have been favorably impressed with the great care exercised by her in making cultures of the skin. Naturally this is a consideration of first rate importance, and errors doubtless account in large part for the numerous organisms described from time to time as the etiologic agents of psoriasis.

At the Institute we have been mostly impressed by the frequency with which Miss Marcus has found sarcinae in psoriasis and its infrequency in control cases, many of whom presented eczematous lesions favorable for bacterial growth. It is necessary, however, to reserve all opinion of the possible relationship of sarcinae to psoriasis and particularly since minor morphologic and cultural differences from other sarcinae are of very little importance. The work is, however, deserving of proper attention, and it is to be hoped that the investigation will be continued to final conclusions.

Dr. John A. Kolmer (again discussing Miss Marcus' paper).—I would sound a note of warning against hasty conclusions of the value of dietary or other forms of treatment in this disease. Dr. Schamberg, Dr. Raiziss, Dr. Ringer, and I found, some years ago, that psoriatics may show marked disturbances of nitrogen metabolism and that a reduced protein intake sometimes favorably influences the clinical cause of the disease, but it is characteristic of psoriasis that periods of temporary improvement are followed by exacerbations which may readily lead to erroneous conclusions regarding any particular method of treatment.

Miss Mary Marcus (closing).—I agree with Dr. Burnett when he says that there is an element of metabolism in the causation of psoriasis. There is no doubt that there is very frequently an improvement in the condition of the patient following the elimination of certain food factors. For that matter, the disease may often disappear temporarily without any change in the diet whatever. The question is not how we can treat the disease palliatively and temporarily, but how can it be cured effectively and permanently?

In reference to the remarks of Dr. Martha A. Wood, it is not quite clear to me when she says: "He (the patient) was put under treatment for the intestinal condition and was cured," whether she meant to infer that the intestinal condition was cured or that the psoriasis itself was cured. Even if the latter was meant, it would not at all prove anything, because of the fact that only one case was treated and even that case was not followed up.

What I am anxious to emphasize, however, is that after a great deal of work along these lines, treating the patients by inoculations with the pure cultures of sarcinae and the filamentous organism recovered from the lesion of psoriasis, I have succeeded in clearing up five cases. Of course, I am still continuing my experiments in a number of cases, and I intend to follow them all up to prove to my own satisfaction, as well as to the satisfaction of the profession, whether or not the results are going to be permanent.

In my report to the New York Dermatological Society on December 16, 1925, I have taken pains in stressing this, to me, very important question of the existence of the filamentous organism, associated with the sarcinae in psoriasis. These organisms I have repeatedly demonstrated bacteriologically in culture and in the tissue itself.

In conclusion, I wish to ask all the coworkers along these lines to be good enough to communicate with me in reference to any results they may have obtained or any information that they can possibly furnish me with reference to this work, in which I am extremely interested.

This is but a preliminary report and far from complete.

LABORATORY METHODS

THE RAPID DETERMINATION OF ALBUMIN IN URINE*

By F. B. Kingsbury, Ph.D., Charles P. Clark, M.D., Gertrude Williams, M.S., and Anna L. Post, A.B., New York City

THE need for an accurate, rapid method for determining albumin in the urine in the smaller amounts, that is, from 0.01 per cent to 0.1 per cent, is quite apparent to anyone who has had occasion to examine urinalysis reports. Heretofore the albumin estimation has been made by the Heller's ring test or by the heat and acetic acid coagulation test under conditions which were not necessarily optimum, and the results were recorded as "slight trace," "trace," "+," "++," etc. These terms are subject to great variation in meaning since they are based upon methods which are not quantitative and which give ample opportunity for great differences in interpretation between individual analysts.

In 1914 Folin and Denis[1] described a method for the quantitative determination of albumin in urine based on the turbidity produced by the precipitating action of sulphosalicylic acid on this substance and comparing a turbidity so produced with that made under the same conditions with a standard protein solution. In 1922 Folin[2] simplified the original method by carrying out the operation in graduated test tubes. Ten urines were treated with sulphosalicylic acid reagent simultaneously with the necessary number of albumin standards, 5 or 6, similarly treated. The estimations were then made by a side by side comparison of known with unknown.

This method was successfully used in this laboratory for more than a year, but it was seen that the repeated preparation of sets of tubes from the various serum protein standards for each ten or twenty urine specimens, involving as it did the obtaining and analyzing of sheep serum with its subsequent careful dilution, would prevent any extended application of this otherwise suitable method. On this account it was undertaken to devise standards which would be permanent with which to replace the freshly precipitated serum standards. The purpose of this paper is to describe the preparation and use of such standards and thus to make possible the extension of this valuable method.

During our work we found that the original dilution at which the turbidity was produced, that is, 1 c.c. of urine or albumin standard solution diluted to 25 c.c. with 2 per cent sulphosalicylic acid was less adequate in determining the lower levels of albumin, 0.01 to 0.05 per cent, than a dilution of 2.5 c.c. of urine to 10 c.c. with 3 per cent sulphosalicylic acid. The latter dilution was accordingly adopted in all the work reported in this paper.

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*From the Biochemical Laboratories of the Metropolitan Life Insurance Company, New York.

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The first attempts to make permanent standards showed that it was desirable to use suspensions, the particles of which were nearly the same size as those causing the turbidity in the average albuminous urine.

For this reason we tried to use the albumin-sulphosalicylate dispersions themselves as permanent standards, but, since the particles of such suspensions always formed larger aggregates, thus materially changing the turbidimetric value of the standard even in the presence of protective colloids, this type of standard with water as the suspending medium had to be discarded. It was necessary, therefore, to use solid or semisolid media, and 10 per cent gelatin solutions were found adequate for this purpose. The material was suspended in warm 10 per cent aqueous gelatin solution, a small amount of formaldehyde added, and the solution allowed to gelatinize by cooling. Such gels, under the action of formaldehyde hardened to such a degree that within a few days they would not melt at any room temperature.

The substance which we found most satisfactory for suspending in gelatin was discovered by accident when testing the preserving action of hydrazin sulphate and hexamethylenetetramine in the same specimen of urine (Kingsbury and Williams). An amorphous white material closely resembling the protein sulphosalicylate formed when an albuminous urine was precipitated by sulphosalicylic acid was noted in the sediment of this urine. We then determined the best conditions for preparing this substance from pure solutions of hydrazin sulphate and hexamethylenetetramine and found that equal volumes of aqueous 1 per cent solution of the former and 10 per cent solution of the latter gave the most suitable product.

In looking up the literature it was found that Pulvermacher[3] had made a compound in 1893 from formaldehyde and hydrazin hydrate with which this is probably identical. We have abbreviated *formalazin*, Pulvermacher's name for this compound, to *formazin*. Elementary analyses were made for us by S. J. Dobrovolny under Dr. W. H. Hunter's direction at the University of Minnesota. (Table I.)

TABLE I

	FORMAZIN, (HEXAMETHYLENETETRAMINE, + HYDRAZIN SULPHATE) KINGSBURY AND WILLIAMS			FORMAZIN, (HYDRAZIN HYDRATE + FORMALDEHYDE) PULVERMACHER
	I	II	III	I
Carbon	41.85%	40.28%	38.85%	42.3 %
Hydrogen	7.84%	7.41%	7.08%	7.11 %
Nitrogen	48.76%	46.66%	46.60%	50.06 % 49.85 %

Formazin is difficult to purify and the samples sent to Dr. Hunter were not as uniformly pure as we could have wished, which accounts for the slight discrepancies in the analytical figures. It seems reasonable to believe that, irrespective of the methods of preparation, the two substances are chemically identical, although physically quite different, for, made by Pulvermacher's directions, formazin is a gelatinous substance resembling aluminum hydroxide cream and cannot be used for our purpose, that is, suspending it in gelatin in such a way as to resemble closely a dispersion of urinary albumin sulphosalicylate. In our method of making formazin the main factor appears to be

the mild acidity, a P_H of about 6.3, which produces a mixture with a constantly low concentration of formaldehyde. Formazin is therefore formed very slowly, and a suitable product is so obtained.

Formazin, as we make it, can be suspended in close imitation of a urinary albumin sulphosalicylate dispersion in the following media: water, glycerin, ethyl alcohol, lactic acid, glacial acetic acid, amyl alcohol, oleic acid and paraffin oil. In this respect it is the most remarkable substance with which we are familiar. The only medium, however, which we have found in which formazin can be suspended and retain its original degree of turbidity unchanged, is a gelatin gel; at least this is the only one that has proved adequate in our hands.

PREPARATION OF GELATIN-FORMAZIN STANDARDS

1. *Preparation of the Gelatin Solution.*

The grade of gelatin which we have found best suited is the Super X brand of the Coignet Chemical Products Company, 17 State Street, New York City. The sheet form is more easily brought into solution than the granulated. Fifty grams of sheet gelatin are placed in a large beaker with 350 c.c. of distilled water. Working time will be saved in bringing this into solution by allowing it to stand over the preceding night in the incubator at 38° C. It is then dissolved by heating in a water-bath at 45° to 50° C. and made up to 500 c.c. with distilled water in a graduated cylinder. The white of one egg is now stirred in and the mixture heated in a boiling water-bath for at least an hour. A clearer product will be obtained if the coagulated egg white is not broken up by stirring. The gelatin solution is filtered hot through a Whatman's No. 4 filter paper, the large clots of albumin also serving as a filter, and allowed to cool. When cool it is put on ice until used. The perfectly clear gelatin gel so prepared must be used within a few days, since no preservative has been found which does not interfere in the subsequent steps of the procedure.

2. *Preparation of the Formazin Suspension.*

Two and one-half grams of hexamethylenetetramine* (urotropin) are dissolved in 25 c.c. of distilled water at room temperature. One-fourth gram of hydrazin sulphate* is likewise dissolved in 25 c.c. of distilled water at room temperature, and the two solutions are mixed in a small flask by pouring the hexamethylene solution into the hydrazin sulphate solution. The flask is then stoppered. In the course of two or three hours the clear, colorless solution becomes turbid and fifteen to eighteen hours later, when the reaction has come to an equilibrium, is ready for use. There will then be a considerable quantity of amorphous white precipitate present which will be uniformly suspended on gently inverting the flask a few times. We have found that the turbidimetric value of this suspension is constant and that it may be used for a period of some months, provided that it is kept stoppered.

*These chemicals and sulphosalicylic acid may be obtained from the Research Laboratory of the Eastman Kodak Co., Rochester, N. Y.

3. *Preparation of the Standards.*

Immediately before using, the 10 per cent gelatin gel is warmed to about 40° C., which liquifies it, and 0.3 c.c. of formalin (40 per cent formaldehyde solution) is added to each 100 c.c. and thoroughly mixed in. Care must be taken not to add an excess of formalin, as this will cause a gradual increase in the turbidity of the standards in the course of time which will render them useless. The 100 mg. standard, equivalent in turbidity to that produced when the corresponding or 0.1 per cent albumin standard is precipitated with three volumes of 3 per cent sulphosalicylic acid, is made by adding 14.5 c.c. of the freshly agitated formazin suspension to 100 c.c. of 10 per cent gelatin solution. This operation is carried out at 40° to 45° C., and care is taken to make the mixing thorough. The stock 100 mg. standard is then diluted with 10 per cent gelatin solution to make the lower standards. (Table II.)

TABLE II

FORMAZIN-GELATIN SUSPENSION, EQUIVALENT TO 100 MG. ALBUMIN PER 100 C.C.	10% GELATIN	STANDARD PRODUCED
25 c.c.	26 c.c.	0.05% or 50 mg.
20 c.c.	30 c.c.	0.04% or 40 mg.
15 c.c.	35 c.c.	0.03% or 30 mg.
10 c.c.	40 c.c.	0.02% or 20 mg.
5 c.c.	45 c.c.	0.01% or 10 mg.

Each standard when made is poured into an appropriate test tube, sealed carefully with a waxed stopper and the top paraffined. In very hot weather it is best to keep the standards in a cool place for a few days, after which the gelatin has hardened to such a degree that it will not melt at any room temperature.

It is essential that the test tubes which contain the permanent standards should have the same internal diameter and wall thickness as those used in the actual precipitation of the urines.

Pyrex test tubes have been found wholly satisfactory, but soft glass tubes may also be used and are easier to obtain in quantity in uniform bore. The internal diameter of the tube should be 12 to 14 mm. A convenient length of tube is 125 mm. It is necessary that all tubes be fairly uniform in bore; that is, should not vary more than 0.5 mm. Pyrex tubes of uniform inside and outside diameters with these diameters etched on the tube to facilitate replacements in case of breakage, with or without the graduations at the 2.5 and 10 c.c. levels may be obtained from R. G. Cargille, 74 Cortlandt Street, New York. Tubes of the softer glass, 12 mm. internal diameter and varying by not more than plus or minus 0.1 mm. have been in use in this laboratory for some time and may be obtained from the Emil Greiner Company, New York, or the Arthur H. Thomas Company, Philadelphia.

The data given above for the preparation of permanent gelatin standards was obtained by making comparisons with standard serum protein solutions precipitated by adding 7.5 c.c. of 3 per cent sulphosalicylic acid to 2.5 c.c. of the protein solution. At the end of ten minutes the turbidity in the protein

standard reaches its maximum. It is necessary to allow this time for consistent results. We have always made use of three serum proteins standards for each gelatin standard. For instance, to make our 50 mg. gelatin standard as accurately as possible, we compare it with the 45, 50 and 55 mg. serum protein standards, selecting that dilution of gelatin-formazin which is closest to the 50 mg. tube. The comparisons are made in the Clark* lamp. This lamp is not absolutely essential but is desirable, since the comparisons are more easily made with it than by daylight. The tubes are lighted nephelometrically, but a strip with a black line at the bottom of the rack enables the observer to make the comparison by transmitted light, or turbidimetrically. When daylight is used it is necessary to hold the tubes in front of a window, and a black background must be provided. This is simply done by attaching such a background to the back of the rack. *Photographs of this rack and Clark's lamp are shown.* (Fig. 1.) All the values for albumin by the turbidimetric or nephelometric method were obtained with Clark's lamp.

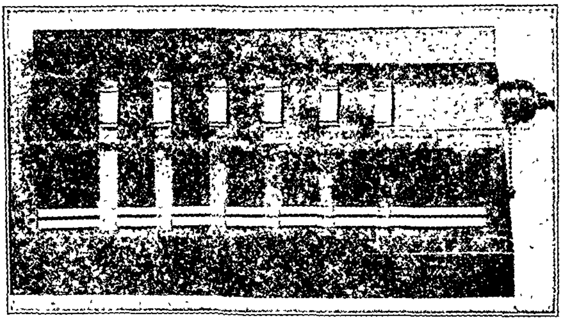

Fig. 1

THE DETERMINATION OF ALBUMIN

Two and one-half c.c. of urine are pipetted† into a test tube, 3 per cent sulphosalicylic acid added from an aspirator bottle to the 10 c.c mark, the contents mixed by inverting the tube, but not by shaking, and the tube allowed to stand *ten minutes. It is then compared with the formazin-gelatin standards. This is best done by arranging the standards in the order of their values in the test* tube rack, leaving one hole between each two standards. It will be found that

*Clark's Lamp may be obtained from Dr. Charles P. Clark, Mutual Benefit Life Insurance Co., Newark, N. J.

†An ordinary Mohr 5 c.c pipette graduated in tenths of cubic centimeters or a special pipette (Fig. 2) of the shape devised by Folin and modified to meet the needs of this laboratory may be used for this purpose. The special pipette has a capacity of about 7 c.c., is graduated 0 at the top, then 1 c.c and finally at 3.5 c.c. One c.c of the urine is drawn off for the sugar determination, 2.5 c.c. or to the 3.5 c.c. mark for the albumin determination, and the rest of the urine, about 3.5 c.c., returned to the original specimen bottle. Without rinsing or practical need to rinse, the next urine is drawn up into the pipette and the measuring process repeated. Although the two samples of urine are measured in the upper portions of the pipette, the actual samples delivered are from the bottom portion or that part freshly rinsed by the same urine in the process of filling the pipette. These pipettes, according to the above specifications, may be obtained from the Emil Greiner Co., New York City.

been made. Such dark-field studies show that in number and size of particles both standards appear very similar, in fact it is practically impossible to distinguish one from the other. While comparisons of this kind between actual urine tests and the corresponding formazin-gelatin standards show practically the same close resemblance in the majority of cases there are others in which the particles of the albumin sulphosalicylate in the urine. appear to be of smaller dimensions than those of the formazin-gelatin and the freshly dispersed serum protein standards.

It should be noted that the color of the formazin-gelatin standards is similar to that of the urine precipitated with 3 per cent sulphosalicylic acid in the proportion used. This makes the turbidity comparison easier than is the case when precipitated sheep serum standards, which are white, are used against the slightly tinted urine tests.

We have compared the values obtained by the use of formazin-gelatin standards with those obtained gravimetrically by heat coagulation. The method we used for this, while similar to that described by Folin and Denis[1] as a check method, differs from it in that we coagulate the albumin at or near its isoelectric point, P_H 4.7, by adding sufficient sodium acetate-acetic acid buffer solution to produce this result. A few drops of 0.02 per cent methyl red is used as indicator.

In Table IV are shown some determinations by this method of serum standards and albumin free urines to which serum protein in known amount had been added.

TABLE IV

	FOUND BY THE MODIFIED GRAVIMETRIC METHOD MG. PER 100 C.C.
First serum standard, contains 100 mg. protein per 100 c.c. as shown by the total nitrogen determination	100
Second serum standard, also made to contain 100 mg. protein per 100 c.c.	94
Albumin free urine to which serum protein had been added to make it 50 mg. per 100 c.c.	53
Albumin free urine to which serum protein had been added to make it 200 mg. per 100 c.c.	201

In Table V are shown some comparative figures obtained with formazin-gelatin standards and the modified gravimetric check method. The urines were not selected except to contain at least 20 mg. albumin per 100 c.c. The formazin-gelatin standards used were equivalent to the following amounts of protein in milligrams per 100 c.c. of solution: 10, 20, 30, 40, 50 and 100. The values given are those of the nearest standards. The plus and minus signs indicate that the values although closest to the standard given are slightly above or below it.

It will be noted that the sulphosalicylic acid method with formazin-gelatin standards agrees well with the gravimetric method when one remembers the inherent difficulties in any nephelometric method and the difficulty of completely removing, purifying and weighing the very small amounts of albumin actually dealt with in the gravimetric method.

TABLE V

LABORATORY NUMBER	BY FORMAZIN-GELATIN STANDARD, MG. PER 100 C.C.	BY THE MODIFIED GRAVIMETRIC METHOD MG. PER 100 C.C.
8153	50	40
8165	20+	18
8134	100–	93
8175	50	37
8285	50–	44
8228	30–	26
8283	50+	53
8356	30+	30
8386	40–	36
8388	40+	36
8404	30–	32
8411	50–	42
8428	100–	71
8454	50	43
8457	100	101
8475	50–	46
8505	40–	39
8521	40+	45
8546	50+	60
8591	30–	21
8617	40+	32

CONCLUSION

An improved sulphosalicylic acid nephelometric or turbidimetric method with permanent standards is described. This method is of sufficient accuracy for use in the collection of data in the routine examination of urines for the smaller amounts of albumin.

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SIMPLIFIED TECHNIC FOR THE SHAFFER-HARTMANN METHOD FOR BLOOD-SUGAR ANALYSIS*

By E. P. Bugbee, M.D., and A. E. Simond, B.S., Detroit, Mich.

After having done several thousand determinations of glucose in standard solutions and in blood we have adopted some modifications of the Shaffer-Hartmann method.[1] These modifications do not introduce errors which are appreciable, in view of the fact that the original method is subject to errors of 10 per cent or more, depending upon the technic of the analyst.

Formerly we standardized our sodium thiosulphate solution against pure iodine or iodine liberated from potassium iodate by the addition of potassium iodide and sulphuric acid. Later we made use of the modification introduced by Haskins and Holbrook.[2] In this modification the thiosuphate is standardized against the Shaffer-Hartmann copper reagent. It is then adjusted so that the thiosulphate instead of being exactly N/200 is of such strength that 19.50 c.c. are required to decolorize 5 c.c. of the copper reagent. This is a great labor saving modification in two ways: (1) it makes the preparation of the thiosulphate much easier, and (2) it makes possible the use of tables or graphs reading in terms of c.c. of thiosulphate used in titration, rather than difference between the blanks and thiosulphate used.

If pure chemicals, carefully prepared solutions, and uniform technic are used, the method of Haskins and Holbrook gives results which are accurate enough for clinical purposes. We demonstrated this fact by doing analyses of a series of standard glucose solutions. Three or four determinations were done on each standard solution. The percentage of error is shown in Table I.

Table I

Error Made in Analyses of Glucose Solutions by the Method of Haskins and Holbrook

Glucose Solution	Analysis	Error	Glucose Solution	Analysis	Error
%	%		%	%	
Blank	0	0	.225	.225	0
.010	.003	–70%	.250	.245	–2%
.020	.006	–70%	.275	.268	–2.5%
.030	.017	–43%	.300	.292	–2.6%
.050	.044	–12%	.325	.305	–6.1%
.075	.068	– 9%	.350	.340	–2.9%
.100	.093	– 7%	.375	.380	+1.3%
.125	.120	– 4%	.400	.380	–5%
.150	.149	–0.7% and –2%			
	.147				
.175	.168	– 4%			
.200	.197	–1.5% and –0.5%			
	.199				

It will be seen by consulting Table I that the errors are enormous for very dilute glucose solutions. For this reason no dependence should be placed upon figures obtained by this method for glucose solutions or blood sugar more dilute than .04 per cent.

Formerly, when doing blood-sugar analyses in dogs, we drew about 3 c.c.

*From the Research Laboratory of Parke, Davis and Company.
Received for publication, November 28, 1925.

of blood for each determination. This was put into a bottle containing two drops of 10 per cent potassium oxalate. Then exactly 2 c.c. of this oxalated blood was pipetted out and added to the water, sulphuric acid and sodium tungstate, according to the Folin-Wu[3] method for preparing the clear protein-free filtrate. When we worked with rabbits we found difficulty in obtaining enough blood, so we tried to get along with smaller samples. We found we

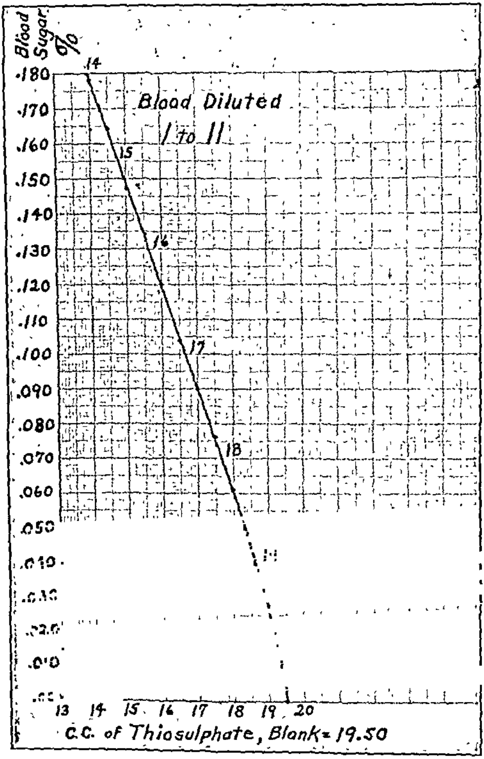

Fig. 1.—Shows per cent of blood sugar in blood diluted 1 to 11 corresponding to c.c. of thiosulphate used in titration.

could draw the blood directly from the rabbit's heart by means of a well oiled 1 c.c. tuberculin syringe. If the heart is pierced near the apex it causes no trouble, but if the base of the heart or the large vessels are pierced, the animal is liable to bleed to death. A tuberculin syringe is quite accurate, and if the same syringe and needle (22 gauge 1¼ inches long) are used for each sample any error is constant.

Haden[4] recommended adding 1 c.c. of blood directly to 8 c.c. of N/12

sulphuric acid and then adding 1 c.c. of 10 per cent sodium tungstate to precipitate the protein. This shortens the process to the use of two solutions instead of three and apparently works just as well.

We found difficulty in obtaining 5 c.c. of filtrate when only 1 c.c. of blood was used. We wished to use as large a volume of the filtrate as possible so as to reduce errors proportionately. We were able to do this by adding another

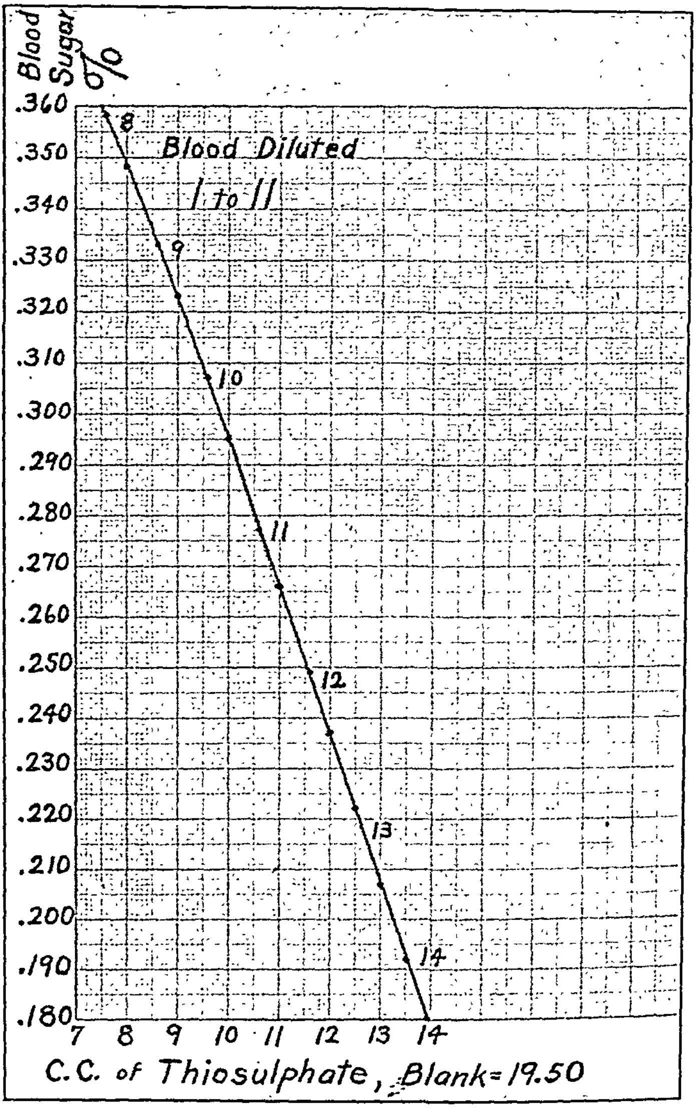

Fig. 2.—Shows per cent of blood sugar in blood diluted 1 to 11 corresponding to c.c. of thiosulphate used in titration.

1 c.c. of water, which made our dilution 1 to 11 instead of 1 to 10. We constructed tables and graphs based on this dilution so that it made the final computation of blood-sugar percentage no more difficult. The extra 1 c.c. of water was combined with the sulphuric acid so that now we use 9 c.c. of 2/27 N H_2SO_4.

We found that it is advisable to do determinations on standard sugar solutions and on blanks at frequent intervals so as to check up on the reagents

as well as on our technic. Sugar solutions may be diluted 1 to 11 instead of 1 to 10 so as to use the same graph as is used for blood sugars.

In constructing our table and graphs we have made use of the figures obtained by Shaffer and Hartmann,[1] because they used purer solutions of glucose than we had at hand. As Haskins and Holbrook[2] also used these figures, our table and graphs may be most conveniently constructed by multiplying the figures given by Haskins and Holbrook by 1.1. Our graphs, Fig. 1 and Fig. 2, are made by drawing smooth curves through points representing values of blood sugar corresponding to the number of cubic centimeters of the sodium thiosulphate used in titration. For directions regarding the method of doing the analysis and for preparation of the solutions reference should be made to the article by Haskins and Holbrook.[2]

SUMMARY

The following modifications of the original Shaffer-Hartmann method for blood sugar have proved to be advantageous.

1. Use of thiosulphate standardized against the copper reagent so that 19.50 c.c. of thiosulphate is required to decolorize 5 c.c. of the copper reagent, as recommended by Haskins and Holbrook.
2. Drawing blood directly into a well oiled 1 c.c. tuberculin syringe so as to do away with the necessity of using anticoagulants and pipettes.
3. Delivery of the blood from the syringe directly into dilute sulphuric acid, as recommended by Haden.
4. Diluting the blood to 1 to 11 instead of 1 to 10 so as to get 5 c.c. of filtrate.

REFERENCES

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[3]Folin, O., and Wu, H.: Jour. Biol. Chem., 1919, xxxviii, 81.
[4]Haden, R. L.: Jour. Biol. Chem., 1923, lvi, 469.

A METHOD OF SKIN SCARIFICATION

By Evans B. Wood, M.D., Atlanta, Ga.

An ordinary dental burr will be found to be a very satisfactory instrument for scarification in making skin tests for sensitization, von Pirquet, smallpox vaccination, and like procedures. Scarification is accomplished by grasping the dental burr between the thumb and index finger, resting the cutting end of the instrument on the skin to be scarified, and giving the instrument a twist.

The instrument is easily sterilized either by flaming or by boiling. It is easily procured and very inexpensive. Various sizes may be had from any dental supply house

This method of scarification has the advantage of producing a uniform area of scarification; the process is almost painless; children are not frightened, and there is little danger of producing any accidental injury. The time required for vaccination is reduced to a minimum when large numbers have to be handled.

HYDROGEN-ION TITRATION OF MEDIA AND PREPARATION OF COLOR STANDARDS*

By B. W. Culyer, Ottawa, Canada

After experimenting with various methods[1, 2, 3, 4] with more or less success, the following technic for the determination of hydrogen-ion concentration as applied to bacteriologic media, has, for the past few years, given every satisfaction in this laboratory. Upon the completion, a year or two ago, of some work on the buffer indices of bacteriologic media, following the technic of Brown,[5] it was thought that his method of titration might easily be adopted as a routine procedure for the titration of culture media. After the initial difficulties experienced in the general preparation of buffer solutions and colorimetric standards have been overcome, this method of Brown, so clear cut and simple, will, I am sure, appeal to most laboratory workers as worthy of general adoption.

METHOD OF TITRATION OF MEDIA

I. *Equipment.*—

a. A selected set of colorimetric hydrogen-ion standards is taken; the following series will cover the ranges necessary for all ordinary culture media:

Brom Cresol Green	4.2–6.0
Brom Thymol Blue	6.0–7.6
Phenol Red	6.8–8.4
Cresol Red	7.2–8.8

b. Tubes of nonsoluble glass, graduated to 10 c.c. and of the same size as those containing the standards. Tubes specially designed for this purpose, as well as tubes for colorimetric standards, already drawn out for sealing, may now be obtained from the Arthur H. Thomas Co., of Philadelphia.

c. A comparator block.

d. Bottles containing the indicators.

e. A dropping pipette with rubber teat and of the same bore as that used in the preparation of the standards.

f. Normal and twentieth normal solutions of NaOH and HCl.

g. Distilled water.

h. Very accurate pipettes of 1 c.c. capacity, and of 0.1 c.c. capacity graduated in hundredths.

II. *Titration.*—

a. Into each of 2 tubes place 1 c.c. of the medium to be titrated and fill up to the 10 c.c. mark with distilled water. It is to be noted that, if titrating agar, the distilled water should previously be made quite hot to allow for a true distribution of the medium in the fluid. Before a reading is made after the contents are well mixed, the tube should again be cooled.

*From the Laboratory of Hygiene, Department of Health, Ottawa, Canada.
Received for publication, February 6, 1926.

b. To one of the tubes (Tube 1) add the same number of drops of indicator as was used in the preparation of the standards chosen for the titration. The other tube containing the medium (Tube 2) is used as a correction screen.

c. Place in the comparator block the standard of the P_H value required; in front of this, place the correction screen (Tube 2); the unknown (Tube 1) is placed alongside and is backed by a tube containing distilled water. A reading will now determine whether the addition of acid or alkali is necessary to obtain a color match. If the unknown does not now match with the standard, either N/20 NaOH or N/20 HCl, as the case may be, is cautiously run into the tube by means of a 0.1 c.c. pipette graduated in hundredths, mixing well after the addition of each drop, until a perfect color match is made. The amount of N/20 used, if multiplied by 5, will give the percentage of a normal solution required to bring the bulk of the medium to the desired P_H value. After addition of the requisite amount of normal acid or alkali to the medium, the test is repeated until finally the sample shows no need of further adjustment.

THE PREPARATION OF COLORIMETRIC HYDROGEN-ION STANDARDS

The buffer solutions and the stock indicators are prepared according to the method and technic of Clark.[6] It is possible to keep the buffer solutions in good condition over a long period by storing in pyrex glass bottles which are pasteurized and sealed with "Viscose" bottle caps. From these are made up the sets of colorimetric hydrogen-ion standards. The procedure is as follows:

A sufficient number of the kind of tubes already specified, enough to accommodate a series within the range of the indicator selected, is placed in a rack. These tubes are to be cleaned carefully, sterilized by heat, and into each is measured accurately, by means of a certified pipette, 10 c.c. of each buffer combination within the range required. A clean, dry pipette should be used for each operation. The next step is the addition of the indicator. This is added by means of an ordinary dropping pipette with rubber teat; several of these, each of exactly the same bore, should be at hand, so that, in case of an accident in the middle of the operations, the pipette may be replaced without variation in the size of the drops. The same number of drops are added to each tube, starting at 5 and working up drop by drop to each, until the point is reached where the desired degree in coloration is attained. The number of drops used should be recorded and later placed on the labels of the standards. With some indicators 7 drops have been found sufficient; with others, as many as 14 drops have been used. The tubes are then placed in a block for comparison; each tube with the next higher and the next lower P_H value. For this purpose a comparator block having three slots for tubes has been found useful. Starting at the lower end of a series, after checking the first three tubes, the left-hand one is removed and the remaining two moved over to the left. Then the fourth tube is placed in the vacant slot and so on. Any error, either in the preparation of the buffer solutions, or in general technic will soon become apparent. If the grading of the series is found to

be correct, the tubes are then sealed off in a flame and heated in a water-bath for thirty to forty minutes at 75° to 80° C., as a precaution against the development of moulds, which, if they do not alter the P_H and thus the color of the standards, render it difficult to make clear-cut readings. After being labelled, the colorimetric standards are stored in a cabinet specially constructed with racks and made to exclude light.

ADVANTAGES OF THIS METHOD

1. By this method, the titration of any highly colored medium is rendered relatively easy and accurate, owing to the high dilution of the medium.
2. The method allows of titrating agar in a liquid, or at least in a highly dispersed state.
3. It is possible, in the majority of instances, by using one tube only, both to establish the primary reaction and to adjust it to the desired end-point.
4. Any reading when multiplied by 5 is in direct terms of N/1 per cent.
5. By using tubes of nonsoluble, clear, white glass and by pasteurizing after sealing, the standard tubes may be kept in perfect condition, practically indefinitely.

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[5]Brown, J. H.: Jour. Bacteriol., November, 1921, vi, 555.
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ALARM APPARATUS FOR ARNOLD STERILIZER*

BY JOHN W. CHURCHMAN, M.D., AND LOUIS SIEGEL, NEW YORK CITY

IN EVERY laboratory in which Arnold sterilizers are used, annoyance and often expense result from the fact that the apparatus, through inadvertence, is allowed to boil dry. To prevent this accident the following simple device has been installed in our laboratory. Since it has proved its value by successful operation for some time, it appears worth while to describe it.

The apparatus requires few parts and these can be purchased in any electrical supply store. It is inexpensive, costing approximately as follows:

1 Piece asbestos board	$ .10
2 Dry batteries	.70
1 Electrical bell	.45
1 Rubber ball	.10
3 Feet insulated bell wire	.03
2 Feet cotton thread	.01
	$1.39

*From the Laboratory of Experimental Therapeutics, Cornell University Medical School.
Received for publication, February 7, 1926.

The apparatus consists essentially of an open electrical circuit, connected to a bell, and a gravity float on the surface of the water in the Arnold. In Fig. 1 the alarm apparatus is shown in solid lines, the Arnold sterilizer in broken lines. The alarm apparatus is placed on top of the Arnold. The batteries should stand on a piece of asbestos to protect them from the heat.

The two batteries (*A*) and (*B*) are connected in series so as to get 3 volts, and one terminal is connected to one binding post on the electrical bell

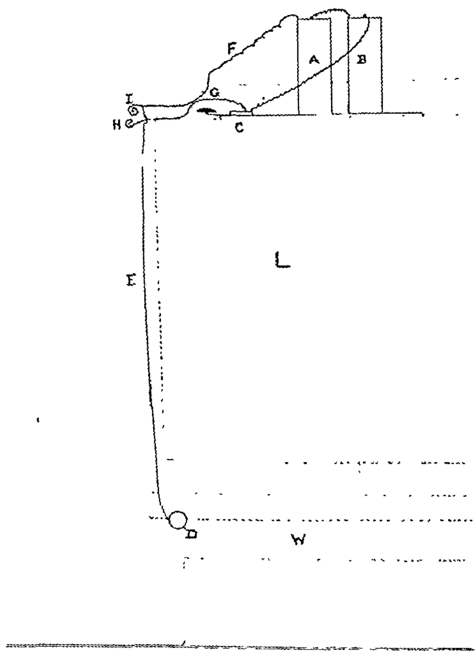

Fig 1.—*L*, Arnold sterilizer; *W*, water in Arnold; *D*, rubber ball float, *E*, thread (a curve has been drawn in thread *E* near wire *G* to indicate that the thread must be so arranged as not to touch the wire, *A*, *B*, batteries, *C*, electric bell, *F*, wire from battery *A*; *G*, wire from bell; *I*, coiled contact surface, wire *F*, *H*, coiled contact surface, wire *G*. The batteries have been indicated in the upright position for the sake of clarity, it is actually more convenient to lay them on their sides.

(*C*). To the other terminal of the battery a wire (*F*) is attached. This wire rests on the top of the Arnold and runs for a short distance beyond its edge. To it is attached a thread (*E*) leading to a rubber ball (*D*) which floats on the surface of the water in the Arnold water cell. From the distal end of the wire (*F*) the insulation is removed for a length of about two inches and the uninsulated portion is coiled so as to make a large surface for contact (*I*). From the other terminal of the electrical bell a wire (*G*) is run along the top

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

Wright, A. E.: New Methods, Devices, and Apparatus for the Routine and Research Laboratory. The Lancet, London, Jan. 2, 1926, iv.

The following are described:

Cover-glass Cell: For the display or enumeration of blood cells, bacteria, or bacterial colonies.

A small pellet of vaseline is placed on a slide and melted by gentle heat. While the vaseline is still liquid, a cover-glass held in a pair of forceps is passed through the vaseline edgewise. When the cover-glass thus edged with vaseline is imposed upon a drop of fluid the escape of the fluid is prevented and the device is termed a simple cover-glass cell.

Hinged and Walled Cover-glass Cell:

A glass slide is cleansed and passed through the flame to sterilize it and a cover-glass similarly prepared. With a soft paint brush the cover-glass is painted around the edges with melted vaseline, one edge being untouched to facilitate lifting.

The vaseline should overlap the edges a millimeter or two. The vaseline upon the farthest edge is made slightly thicker to form the hinge.

The cell is formed by placing the cover-glass with a vaselined surface downward on the cover-glass.

When it is desired to fill the cell, the cover-glass is raised by the edge without vaseline and will stand upright upon the vaseline hinge mentioned above. When the cell is filled the cover-glass is lowered and sealed by painting additional vaseline around the edges.

Such hinged cells may be used as bacterial culture chambers, for differential counting of living and dead organisms in a culture or similar observations.

When using such a chamber for bacterial counts the uppermost and nethermost layers of the fluid may be indicated by the following simple device.

A piece of fine emery paper (00) is drawn horizontally along the slide with firm pressure, thus marking longitudinal striae on the slide.

By placing a cover-glass upon the emery paper, pressing down with the finger, and moving the glass about one inch, a similar set of striae may be produced. The glass and slide may be so adjusted that small squares may be formed which facilitate the count and indicate the two delimiting planes.

Cram, E. B.: The Egg-producing Capacity of Ascaris Lumbricoides. Jour. Agricult. Res., May 15, 1925, xxx, No. 10, p. 977.

By means of a laborious and painstaking study it is computed that the total number of eggs, fully developed and in process of development, in the female Ascaris lumbricoides is from 26,000,000 to 27,000,000.

Hull, T. G., and Nauss, R. W.: Laboratory Differentiation of Smallpox and Chickenpox. Amer. Jour. Pub. Health, February, 1926, ci.

As a result of rather extensive observations the following conclusions are formulated:

1. Rabbits immunized with vaccine virus will show, upon intracutaneous injection of vaccine virus or material from smallpox lesions, typical allergic reactions within 48 hours.

2. Serum from chickenpox lesions rarely produces allergic reactions in immune rabbits.

3. Chickenpox material does not sensitize rabbits to further injections.

4. Scabs from chickenpox cases usually cause an inflammation on smallpox immune rabbits.

5. Factors producing inconsistent results are individual variations in the animal and variations in different strains of material.

6. The amount of virus injected directly influences the degree of the reaction.

7. Blood serum from an individual highly immunized to vaccine virus will produce an allergic reaction in immune rabbits.

8. Nonpotent vaccine virus will produce an allergic reaction in immune rabbits.

9. Rabbits lose their immunity in 9 to 14 months.

10. Guinea pigs and rats are not suitable for this test.

DeJong, S. I., and Handuray, P.: Concerning a Case of Typhoid Fever: Late Examination of Haemocultures in Nonbiliary Medium: A New Interpretation of the Electivity of the Biliary Media. Bull. et Mem. Soc. Med. d. Hôsp. d. Paris, xli, 1561.

Absence of growth in bouillon after four days is usually interpreted as a negative result. When growth occurs in a bile medium inoculated at the same time, the broth remaining sterile, it has been assumed that the lack of growth was due to the bactericidal power of the inoculated broth.

DeJong and Handuray report a case in which a bile culture made on the sixth day gave a growth of typhoid bacilli, a culture made at the same time in peptone-water remaining negative.

On the seventeenth day, however, the peptone-water contained motile bacilli, morphologically typhoid bacilli which failed to grow on transplanting.

The filtrate of this culture destroyed fresh cultures of typhoid bacilli but failed to destroy typhoid organisms grown in bile.

The authors extol the advantages of bile for blood culture in typhoid and advance the suggestion that bile may be able to prevent the destructive action of bacteriophage.

Herrold, R. D., and Saelhof, C. C.: Skin Reactions with Filtrate of Koch Strain of Bacillus Tuberculosis. Jour. Am. Med. Assn., March 13, 1926, lxxxvi, 747.

The Koch strain is an avirulent culture giving a profuse growth overnight and originally isolated in 1888.

Studies were conducted to determine if the intracutaneous injection of broth filtrates would give a skin reaction

Skin reactions were obtained in normal adults in dilutions up to 1:50.

The results are tabulated below:

TYPE OF PATIENT	NO.	POS.	DOUBTFUL	NEG.
Advanced tuberculosis	35	0	0	35
Mod. advanced tuberculosis	31	0	0	31
Incipient tuberculosis	8	0	0	8
Apparently normal adults	61	47	8	6
Apparently normal children	14	2	3	9

It is tentatively suggested that the presence of a reaction in the majority of apparently normal adults may indicate that a substance is produced by the growth of this strain in broth which acts in a different way from tuberculin, and that the reaction may mean that, in the presence of an active focus, there may be sufficient antisubstance produced to neutralize the toxic substance.

Smith, J. W., Jr.: Standardization of Typhoid Vaccine. Jour. Infect. Dis., November, 1925, xxxvii, No. 5, p. 385.

Inasmuch as the dosage of bacterial vaccines is customarily expressed in terms of numbers of bacilli per centimeter, the actual enumeration of bacteria becomes the only really practical basic method despite the fact that more accurate methods of standardization are possible and have been proposed.

Smith, therefore, investigated the accuracy of methods of vaccine standardization by bacterial counts.

In the usual Wright method there is a basic error due to the loss of red blood cells accounted for, in large degree, by sedimentation and adherence of the cells to the walls of the counting apparatus and, in lesser degree, to a loss of red cells by hemolysis.

A method of "wet Wright counts" was, therefore, devised as follows:

1. A bacterial suspension is prepared exactly as for the Helber count.
2. Exactly equal volumes of this suspension and the secondary dilution of blood cells are mixed.
3. A drop of this mixture is placed in the Helber chamber and examined.
4. The red cells and bacteria are counted in 40 to 100 small squares.
5. Calculations are made as by the usual Wright method.

In this preparation are obtained a red cell count and a bacterial count.

Even with the "wet method" the same sources of error are encountered though to a lesser degree.

Because of this loss of red cells the ratio of cells to bacteria is not 5:x but 2.5:x or 3:x. Consequently, when the ratio 5:x is employed the bacterial count is overrated and the final suspension is numerically only about one-half as great as it appears to be.

Smith contends, therefore, that the counting chamber (Helber) method is the method of choice and that the expression of dosage should be adjusted in conformity.

Peterson, R. A.: A Simple Blood Coagulometer. China Med. Jour., November, 1925.

The apparatus described consists of a wide-mouthed bottle of 70 to 100 c.c. capacity and about 8 to 12 inches high.

A rubber cork with three perforations carries, in the middle perforation, a clinical thermometer, on either side of which are thrust two inoculating needles each carrying a platinum wire in which four loops have been made, one above the other.

The bottle is filled with water at 100° F. to such a level that the loops clear the water when the stopper is inserted. The loops are filled with blood and one loop at a time thrust into the water until one of the loops remains occluded by fibrin.

The normal coagulation time by this instrument is 4½ minutes.

St. George, A. V., and Brown, A. L.: The Value of the Icterus Index in Differentiating Anemia. Arch. Int. Med., December, 1925, xxxvi, 847.

The authors have modified the Meylengracht method by using his potassium dichromate solution (potassium dichromate 0.05 gm., water 500 c.c., and conc. sulphuric acid 2 drops) as representing 1 and preparing a series of permanent standards of increasing intensity. The necessity for diluting the specimen is thus done away with and the comparison simplified.

From a study of this method in 120 cases they formulate the following conclusions:

1. The normal range of the icterus index falls between 3.5 to 4.5, the average being 4.41 in their series of 120 cases.

2. Rationally interpreted, the icterus index is a distinct aid in the differentiation of anemias, uncomplicated cases of hemolytic anemia having a high range, and uncomplicated secondary anemia having a low range.

3. The severe anemia of carcinoma may be differentiated by its consistently high index (8 to 12.5) from the uncomplicated pernicious anemia which it may simulate.

4. The most common factors increasing the icterus index above that usual for the primary disease are, coexisting pneumonia, cardiac insufficiency and chronic sepsis, eespecially in the biliary system.

The most common factor decreasing the index below the usual level for the primary disease, is hemorrhage, especially a slow, constant bleeding.

5. It is suggested that the icterus index may be used to follow the course of pernicious anemia and to determine, before the blood count falls, when it is advisable to give a transfusion as well as when spontaneous improvement is likely.

Kowarski, A.: A Method for the Determination of Lactose in the Blood. Ztschr. f. klin. Med., Dec. 22, 1925, cii, 382.

The method is based upon the difference between the reduction power of lactose and glucose.

The reducing power of lactose is changed after inversion by boiling with acid; that of glucose remains unaffected. Lactose may be determined, therefore, on the basis of the difference between the reduction power before and after boiling with acid.

Method.—Into a long-necked, flask-shaped, measuring vessel with a capacity of 50 c.c. pour 20 c.c. n/100 acetic acid, 20 c.c. distilled water, and 2.5 c.c. of the serum from the blood which is to be examined. The mixture is placed in boiling water for 1 to 2 minutes, then 3 to 6 drops of n/10 sodium hydroxide added, and the whole again placed in boiling water for five minutes. The albumin coagulates in large clots, and the fluid becomes quite transparent.* The mixture is then allowed to cool, and about 0.5 gm. aluminum silicate added. The flask is filled to its capacity mark, the mixture shaken strongly, and then filtered. The filtrate must be water clear and absolutely free of albumin (control with sulphosalicyclic acid). Then place 20 c.c. of the filtrate corresponding to 1 c.c. of the serum, into each of two large reagent glasses of 50 c.c. capacity. To the first add 1 c.c. 20 per cent sulphuric acid, place the reagent glass in a boiling water-bath, and heat for 1 hour. Then to the contents of the second glass add also 1 c.c. 20 per cent sulphuric acid. To each add 0.4 gm. sodium potassium tartrate, 2.0 c.c. 30 per cent sodium hydroxide, and 2.0 c.c. of a copper sulphate solution which contains sugar (0.1 gm. chemically pure dextrose, 8.0 gm. copper sulphate, and distilled water to 200 c.c.). The salt is then dissolved by stirring, and the reagent glasses heated in the water-bath for five minutes. After the glasses have been cooled for one minute in running water the red oxide of copper which separates out is collected on an asbestos filter and washed. The apparatus required for this is an Erlenmeyer flask which is closed by a two-hole rubber stopper. In one of the holes is an Allihn funnel holding the asbestos filter; and through the other hole the flask is connected with a water pump by a bent glass tube. The stopper with the funnel should also fit an Erlenmeyer flask with a 30 c.c. capacity. The asbestos filter should be made of well-beaten, white, asbestos wool, which is kept suspended in 5 per cent nitric acid. The wool is pressed out with a thick glass rod. The filter is used in such a way that the red oxide of copper is deposited only upon the upper surface, and does not press into the lower layers.

The contents of the cooled reagent glass are poured into the Allihn funnel, and an equal quantity of distilled water added. The flask is then connected with the water pump, and the blue fluid sucked through the filter. As soon as the fluid has passed the asbestos filter the water from the reagent glass is immediately poured into the funnel. (Air suction must be prevented, since this will occasion a further oxidation of the red copper oxide.) Again pour 10 c.c. of water into the reagent glass, and then pour this also into the funnel. Meanwhile, have, in a graduated cylinder, 10 c.c. of an iron sulphate solution (10 gm. ferric sulphate, 40 gm. sulphuric acid, and distilled water to 200 c.c.), and pour 5 c.c. of this also into the reagent glass after the water has been removed. As soon as the washing with water is finished the connection of the flask with the pump is broken, the stopper with the funnel placed in the small Erlenmeyer flask, the fluid from the reagent glass poured into the funnel, and the other 5 c.c. from the graduated cylinder added. The flask is then again connected with the pump, and all the iron sulphate solution passed through the filter. The red copper oxide is completely dissolved by this, and changed into an equivalent quantity of red oxide of iron. The connection with the pump is again broken, and the stopper replaced in the large flask. The filter is now washed with distilled water to remove all the traces of iron sulphate solution from the asbestos. The contents of the flask are titrated with a freshly made solution of potassium permanganate. (Permanent solution contains 1.0 gm. permanganate in 200 c.c. distilled water; titer used is a tenfold dilution of this—5 c.c. to 50 c.c.) The titration is continued until there is a permanent rose color. The burettes used here

*The addition of the sodium hydroxide must be done a drop at a time, because the reaction of the fluid must not become alkaline.

should be graduated in 0.05 c.c. so that quantities as small as 0.025 c.c. can be measured. After the quantity of the potassium permanganate solution used has been noted, the same procedure is repeated with the fluid from the second reagent glass. If the same quantity of permanganate solution is used in both cases there is no lactose present in the blood. If more is used for the tube in which the contents have been boiled with the sulphuric acid, the quantity of lactose per c.c. of blood is found by multiplying the difference by 1.66 mg.

Example: 4.80 c.c. permanganate solution used for the first tube; 5.35 c.c. used for the second tube. The difference is thus 0.55. Multiplied by 1.66 mg. this gives 0.88 mg. lactose per c.c. of blood. That is, 88 mg./100 c.c., or 0.088 per cent.

The amount of dextrose in the blood examined is then determined as follows:

"Multiply the reduction found—in our case 0.55 c.c.—by 10/3. (0.55 x 10/3 equals 1.83). This figure gives the quantity of permanganate which corresponds to the lactose. If this figure is subtracted from the figure which corresponds to the total reduction—in our case this is 5.35—we get the quantity of permanganate used to reduce the dextrose in the blood and in the reagent. (5.35 – 1.83 equals 3.52.) The 2 c.c. of copper sulphate solution in the reagent contains 1 mg. of dextrose. This latter can be determined from time to time in a series of experiments. It remains extraordinarily constant, showing only very small variations of quantities of 2.0 c.c. After the subtraction of this figure, and the multiplication of the remainder by 0.5, we find the quantity of dextrose in mg. per c.c. of blood. In our example the value was 3.52 – 2.0 equals 1.52; 1.52 x 0.5 equals 0.76 mg./c.c. That is, 76 mg./100 c.c., or 0.076 per cent."

The figures computed for the dextrose content are valuable only when a glycolysis has been prevented. Thus, for a simultaneous determination of the dextrose and lactose in the blood, it is absolutely necessary to make the determination immediately after the blood is removed from the patient.

Kendall, A. I.: Intestinal Intolerance for Carbohydrate Associated with Overgrowth of the Gas Bacillus. Jour. Am. Med. Assn., March 13, 1926, lxxxvi, 737.

Kendall describes a syndrome, quantitative rather than qualitative, insidious in onset, and generally associated with some unusual dietary event, though really a culmination rather than a commencement.

The typical symptoms are: constipation, fatigue, meteorism, precordial or hypochondrial pain, sometimes only elicited on deep pressure, hypotension, and intolerance for certain foods.

The etiology is obscure, but gas bacilli, and, less commonly, members of the mucosus group of bacilli of the starch fermenting type are found in unusual numbers which may result in a decrease in the normal number of lactic acid bacilli in the small intestine.

Gilbert, R., and Humphreys, E. M.: The Use of Potassium Tellurite in Differential Media. Jour. Bact., February, 1926, xi, No. 2, p. 141.

A concentration of 1:17,000 of potassium tellurite was found to give excellent results in the isolation of diphtheria bacilli from throat cultures and to have no effect on the virulence of the organisms.

The growth of Gram-positive cocci, certain of the higher bacteria, and yeasts and molds seemed only slightly inhibited, while many of the motile bacilli, including a number of anerobes, were markedly inhibited.

The formula follows:

Beef infusion agar 1.5 per cent	1 liter
Horse serum (sterile)	5 per cent or 50 c.c. per liter
Glucose C. P., 20 per cent solution (sterile)	1 per cent or 10 c.c. per liter
Potassium tellurite C. P., 1 per cent solution (0.58 per cent on test)	1 per cent or 10 c.c. per liter 1:34,000 Te_4 (1-17,000 K_2TeO_3)

Procedure.—Melt the agar and cool to 50° C. Add the serum glucose and tellurite solutions, all previously warmed to 50° C. Mix well and insert a sterile siphon. Dispense in Petri dishes. Incubate the plates and examine for contaminations.

The solution of potassium tellurite is prepared as follows:

Potassium tellurite C. P.	0.25 gm.
Distilled water	25.0 c.c.

Procedure.—Weigh 250 mg. K_2TeO_3 on a chemical balance. Grind ,to a very fine powder in a small mortar and add about 10 c.c. of the water. Mix well, allow the undissolved portion to settle and pour off the supernatant fluid. Add more of the water to the residue, grind again and combine the two portions. Rinse the mortar with the remainder of the water.

The solution is then passed through a paper filter and finally through the Mandler to sterilize it.

Note.—It is difficult to get this compound into solution and even after thorough grinding in the mortar a small amount of fine suspension is visible. Heating gently to about 55° C. aids the action somewhat.

Elwyn, R., and McMaster, P. D.: Studies on Urobilin Physiology and Pathology. I. The Quantitative Determination of Urobilin. Jour. Exper. Med., April, 1925, xli, No. 4, 503.

The method described is one of comparative fluorescence.

Standard fluorescent solution.

Alcohol 60 per cent	2,000 c.c.
Zinc acetate	50 gm.
Concentrated HCl	2 c.c.

Filter till clear.

Standard fluorescent solution.

Acriflavine	1 mg.
Water	30,000 c.c.

This can be made by appropriate dilution of a 1:1000 solution which is kept in a cool, dark place. The standard solution is stable for one week if kept in the dark.

Method.—The urobilin, recovered from the fluids or substances containing it as described below, is dissolved in the alcoholic zinc acetate solution to develop fluorescence and compared in a comparator with the standard fluorescent solution.

To 15 c.c. portions of the standard solution graduated amounts, as 0.5, 0.55, and 0.6 c.c. of the fluorescent urobilin solution are added, the tubes gently shaken and compared with tubes containing 15 c.c. of standard fluorescent solution.

The nearest color match is noted and the dilution value of the solution under test calculated.

If the unknown diluted is too weakly colored, 0.1 c.c. amounts of urobilin filtrate are added until a match occurs. If the color of the unknown is too intense, a preliminary dilution of the zinc acetate solution is made and this dilution factor multiplied with the final value.

Calculation of Dilution Value.

The dilution value of the fluorescent zinc acetate filtrate must always be multiplied by a factor dependent upon the number of dilutions involved. Thus with bile, 8 is the characteristic factor since 20 c.c. of the secretion was used, diluted to 80 c.c. during clearing and the volume doubled in the final acetate filtrate. With urine it is 2 since each specimen is diluted but twice. With stool the factor is always 8. In each case, of course, the value is always multiplied according to the amount of the daily specimen.

Separation of Urobilin.

Urine.—To a portion (25 c.c.) of the 24-hour specimen made neutral or barely acid to litmus, about a gram of dry zinc acetate is added and sufficient alcoholic solution of zinc

acetate to make the volume 50 c.c. Shake and filter till clear. A drop or two of tincture of iodine is added to oxidize all the urobilinogen to urobilin and the further treatment is as indicated above.

Stool.—The total stool is agitated with water immediately after collection until a finely divided emulsion is secured.

Dilutions of 250, 500, 750, and 1,000 c.c. are made in accordance with the amount of the specimen and the estimated urobilin content.

Aliquot portions (25 c.c.) are shaken with 75 c.c. of acid alcohol (95 per cent alcohol, 1,600 c.c., conc. HCl 25 c.c. and water to make 2,500 c.c.) in a mechanical shaker for one hour. *The specimen is then allowed to stand overnight to complete oxidation.*

The next day the mixture is shaken and 25 c.c. portions are taken, about a gram of dry zinc acetate added and alcoholic solution of zinc acetate solution added to make the volume 50 c.c. Shake, filter till clear, and add a drop or two of tincture of iodine. The further treatment is as indicated above.

Bile.—To 20 c.c. add 20 c.c. of 30 per cent ferric chloride solution and 40 c.c. of 20 per cent ammonia water. Filter into a 50 c.c. graduated flask or tube until 25 c.c. are obtained. To this amount of filtrate add concentrated HCl until barely acid to litmus, add about a gram of dry zinc acetate and make up to 50 c.c. with alcoholic solution of zinc acetate. The further treatment is as above.

As the standard fluorescent solution has the same value as 1 mg. of urobilin in 950 c.c., if all dilution values are divided by 950, urobilin content can be expressed in terms of mg. of urobilin.

These methods were devised for experimental work on dogs and their possible clinical application is suggested.

O'Leary, P. A., Goeckerman, W. H., and Parker, S. T.: Treatment of Neurosyphilis by Malaria. Arch. Dermat. and Syph., March, 1926, xiii, No. 3, p. 301.

A preliminary report of a ten months' trial of the method in a series of 35 patients.

Of 24 paretics, 25 per cent had a complete remission two months after the malaria was stopped, 37 per cent were definitely improved, and two died, one as a result of malaria.

There were no serologic changes in the blood or spinal fluid nor any improvement in the objective findings. Optic atrophy, gastric crises, lightning pains, asymptomatic neurosyphilis, and paresis sine paresi were unaffected.

The use of arsenicals, mercury, or tryparsamide immediately after the malaria was attended by relapse from remission.

The relation of the method to public health, the various complications and the methods of forecasting these and so on are discussed.

The authors believe the method merits exhaustive investigation.

Goodpasture, E. W.: A Study of Rabies with Reference to a Neural Transmission of the Virus in Rabbits, and the Structure and Significance of Negri Bodies. Am. Jour. Pathology, November, 1925, i, No. 6, p. 547.

The following technic was used for the demonstration of Negri bodies:

1. Fix fresh tissues in Zenker's fluid for 24 hours. The method is not applicable to tissues fixed in Helly's fluid.

2. Stain sections cut from paraffin 10 minutes in the following solution:

Alcohol 20%	100.0 c.c.
Phenol (pure)	1.0 c.c.
Anilin oil	1.0 c.c.
Basic fuchsin	0.5 gm.

The finely powdered or granular dye dissolves easily and the solution is immediately ready for use. Exposure to light and air causes a precipitation of the dye in about two months so that the solution becomes weaker and must be discarded. It is convenient to

keep the required alcoholic solution of fuchsin, and to add the phenol and anilin oil when it is desired to make up the stain anew. If sections are stained in the solution for an hour or more they gradually become brown and useless. It is safe to stain the sections as long as one-half hour but the best results are obtained within ten minutes.

3. Wash off the excess stain in running water, blot with filter paper and decolorize in 95 per cent alcohol until the sections become pink.

4. Wash off in water and counterstain 15 to 60 seconds with Loeffler's methylene-blue.

5. Wash in water. Dehydrate and decolorize for a few seconds in absolute alcohol (until the excess blue is removed).

6. Clear in xylol; mount in balsam.

By this method Negri bodies are stained a sharp, bright red against a pale blue background.

"InnenKörper" are blue, nucleoli, fibrin, and red blood cells are red and Nissl substance blue.

The smallest forms of Negri bodies are seen.

The investigation demonstrated that:

1. Rabbits can be infected fairly regularly by the injection of street virus into the right masseter muscle.

2. Paralysis first occurs in the fore legs and more often on the inoculated side.

3. It is believed that the virus enters the central nervous system through nerves supplying the inoculated muscle.

4. "Lyssa bodies" seem to be formed directly from a digestive change in the neurofibrillar substances of ganglion cells.

5. Typical Negri bodies seem to be formed within the living cell by a focal degeneration of neurofibrillar material which coheres about one or more of the structures resulting from mitochondrial degeneration.

6. The type of degeneration of mitochondrial substance in axis-cylinders and nerve cells the formation of small bodies staining deeply with Loeffler's methylene-blue after acid fuchsin—is, apparently, specific for rabies.

7. It is believed that neither the virus of rabies nor a constant structural change associated with its pressure has as yet been demonstrated microscopically.

8. The hypothesis is advanced that the degenerative changes observed in axis-cylinders are the result of the passage of the virus along these processes and indicate its manner of extension from one to another.

The material resulting from the degeneration of mitochondria may serve as a favorable medium for the growth and spread of the virus.

The paper is illustrated with five colored plates.

Mill, C. A.: Determination of Small Amounts of Bismuth in the Urine. The Lancet, London, Dec. 19, 1925, ccix, 1281.

The modern therapeutic use of bismuth and its compounds has rendered its detection in the urine of interest and a method has been devised for this purpose by Mill.

1. Destruction of Organic Matter.

Into three separate 100 c.c. silica beakers measure 5, 20, and 50 c.c. of urine and evaporate on an asbestos mat over a Bunsen flame to about 5 c.c.

Remove the beakers and add to each 5 c.c. of nitric acid, (sp. gr. 1.4) replace over the flame and evaporate to dryness, using as low a flame as possible. Continue the heating with a low flame until the organic matter is oxidized but not charred. It is important to stop the ignition when all the nitric acid has been driven off and to cool and moisten again with nitric acid. If necessary the procedure may be repeated until only a white residue remains.

After cooling, add 2 drops of nitric acid and 25 c.c. of distilled water and heat to boiling for one or two minutes and transfer the solution to a Nessler cylinder. Rinse out

the beaker with boiling water, transfer the rinsings to the cylinder and make up to 50 c.c. with boiling water.

The solution should be clear and colorless and filtration will not be necessary if a low temperature has been maintained during the destruction of organic matter.

2. Determination of Bismuth.

To the cooled solution in the Nessler cylinder add 0.3 gm. of urea, dissolve and mix thoroughly, and then add 0.5 gm. of phenazone (antipyrin). Dissolve by shaking and, while shaking, add small crystals (0.05 gm. weight) of potassium iodide one at a time until a red color develops. Then add one crystal in excess.

The color thus produced (due to bismuth iodide) is compared to that of a standard solution prepared as follows:

Dissolve 0.62 gm. of bismuth carbonate (B.P.) in 10 c.c of nitric acid (sp. gr. 1.4) and dilute to 1000 c.c. with distilled water, ten c.c. of this solution is then diluted to 1000 c.c. with distilled water and constitutes the standard.

In the determination, to 10 c.c. of the standard solution (equivalent to 0.05 mg. of bismuth) add 1 drop of nitric acid and dilute to 50 c.c. in a Nessler cylinder. The remaining steps are identical with those of the unknown.

The three unknowns are matched with the standard. The one matching contains in the original amount of urine, 0.05 mg. of bismuth.

McMaster, P. D., and Elman, R.: Studies on Urobilin Physiology and Pathology. II. Urobilin Derivation. Jour. Exper. Med., April, 1925, xli, No. 4, p. 513.

Experiments upon the delivery of bile to the intestine.

Experiments upon dogs led to the conclusion that the existence of urobilin in the stool and bile depends upon the delivery of bile to the intestine.

Urobilin can be obtained from bilirubin by reduction through the action of intestinal bacteria, notably B. putrificus.

The experiments reported indicate that there is a direct absorption of urobilin from the intestine and that it is secreted into the bile through the liver. Urobilin is not directly derived from bleeding into the bowel or tissues.

Mussey, R. D.: Eclampsia, with Unusual Nonprotein Nitrogen in the Blood. Am. Jour. Obst. and Gynec., December, 1925, x, 6.

A case report of a patient, aged twenty-seven, with the clinical symptoms of eclampsia in whom the following blood chemistry was found:

Third day:

Urea	168.0 mg.
Uric acid	18.3 mg.

Fifth day:

Urea	221.0 mg.
Creatinine	14.5 mg.
Uric Acid	14.5 mg.

On the seventeenth day the findings had returned to normal and two months later all tests for renal function were normal.

BOOK REVIEWS

(Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.)

*Surgical Pathology**

IN this volume the authors have attempted to supply a comprehensive survey of surgical pathology in which the embryology, anatomy, pathology, and clinical observations are correlated.

The discussion of each subject follows this plan very successfully and, though brief and to the point, is thorough, so that a large amount of material is included.

The illustrations are taken from specimens in the museum of the Royal College of Surgeons and that of the Charing Cross Hospital Medical School. They are well selected.

The style is clear and readable, and the treatment of the various topics is concise but not ambiguous. The typography and proof reading are excellent.

The volume is primarily a textbook, a dictionary of surgical pathology, and, as such, is a very excellent reference work.

Determinative Bacteriology†

THIS is the second edition of the manual published under the editorial direction of the committee on Determinative Bacteriology of the Society of American Bacteriologists.

This volume needs no introduction to the bacteriologist or clinical pathologist, the first edition having become established as a necessity in any laboratory concerned with bacteriologic examinations.

The revision has been extensive and thorough. The keys have been rearranged and amplified; new species have been added, and many descriptions have been expanded.

Not the least valuable portion of the book is an extensive and excellent cross reference index, including both old and new names, compiled by Robert S. Breed of the New York Agricultural Experiment Station 1.

So numerous and varied have been the changes in bacteriologic nomenclature that this comprehensive index is a valuable and welcome addition to the book. The lack of such an index was felt in the first edition.

*Surgical Pathology. By C. J. Marshall, Lecturer on Surgical Pathology, Charing Cross Hospital Medical School, and A. Piney, Director, Institute of Pathology, Charing Cross Hospital. 173 illustrations. Pp. 478. Price $7.50. D. Appleton and Co., New York.

†[illegible] Bacteriology, a Key for the Identification of Organisms of [illegible] David H. Bergey, Professor of Bacteriology, University of [illegible] eference Index of Old and New Names by Robert S. Breed. [illegible] Williams and Wilkins Co., Baltimore.

As it stands, this is a volume which should be purchased for the daily use of laboratories and bacteriologists. There is none which can successfully supplant it in the particular field which it covers.

It is not intended to present the final word in bacteriologic classification, which, to some extent is now, and must remain for a time, in a state of flux, but it serves as an authoritative presentation of the information thus far available and of practical value, and it lends itself to the evolution of a more satisfactory classification in the future.

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*Intravenous Therapy**

AS THE title implies, this book includes a discussion of all therapeutic efforts brought to bear upon the blood stream. It is presented by the author as a manual of intravenous therapy.

The first section of two hundred pages, after an interesting outline of the history of intravenous therapy, is devoted to a detailed discussion of the technic and indications for saline infusion and the transfusion of blood, and this part of the book provides a comprehensive and useful manual of these procedures.

The remainder of the book is concerned entirely with the indications for intravenous medication, diseases being arranged in alphabetical order.

The immediate impressions gained from a perusal of this section of Dr. Dutton's work are: first, that he is an ardent proponent of the intravenous administration of therapeutic agents, almost, it would seem, to the exclusion of other methods; second, that he has assiduously searched the literature for references to substantiate this cause; and third, that, in the presentation of the material thus gathered, there is no sharp distinction between that which *has been* or *might be* administered intravenously and those agents which it is desirable so to administer. As a consequence, there are few diseases known to man which do not appear in the list presented and for which intravenous treatment is not advocated.

One notes the intravenous use of trypsinized extracts of neoplastic tissue in carcinoma; and the statement that by the injection of a hexymethylenamine solution "formaldehyde is at once liberated in the blood, which, as it is a vigorous and powerful antiseptic, destroys the pathogenic germs wherever they may lurk." This is hardly in accord with the usual conception that dissociation of formaldehyde from this drug requires an acid medium. Other similarly somewhat unconsidered statements are to be found here and there under various headings.

The references consulted are, indeed, numerous; too many, however, relate to a series of "one consecutive case," and the author grants, in his preface, that many come from sources of little authority.

It is unfortunate, also, that references to the use of remedies of unknown composition and known only by trade names occur not infrequently.

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*Intravenous Therapy. By W. F. Dutton, Formerly Medical Director, Polyclinic and Medico-Chirurgical Hospitals; Director, Medical Research Laboratories, Amarillo, Texas. Ed. 2. Pp. 594. 64 illustrations. Cloth. Price $6.00. F. A. Davis Co., Philadelphia.

Therapeusis has, at times, been the creature of the winds of chance. It would be regrettable if this book were to serve as the instigation or warrant for the indiscriminate intravenous administration of remedies.

As a source of reference concerning the applications—scientific, empirical, or experimental—of intravenous therapy the book serves a useful purpose, but it must be taken as a record of what has been done or may be done rather than as a definite statement as to what *should* be done in the field of intravenous treatment of disease.

The second portion of the book cannot be accepted as authoritative or as representative of the accepted medical thought of the day and must be read with a full comprehension of the fact that it represents empirical rather than accepted methods based upon sufficient, comprehensive, or scientific investigations.

It is clearly an attempt to make out a case in which the facts are fitted to the theory rather than the reverse.

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*The Cerebrospinal Fluid in Clinical Diagnosis**

THIS book is an attempt to present in compact form "all that is definitely known about the nature of the spinal fluid and its variations in disease."

After a clearly presented consideration of the anatomy and physiology, followed by a discussion of the nature and composition of the spinal fluid, the authors proceed to a discussion of the various changes which occur in disease. This constitutes Part I of the book.

Part II discusses the spinal fluid findings in meningitis, syphilis, various nervous diseases, and some points of differential diagnosis.

Part III comprises the technic of various methods of examination.

While not exhaustive, the text is well written and informative. The volume serves a useful purpose as a reference work in this field of special pathology, but is neither comprehensive nor cosmopolitan enough in its references to present an all-inclusive survey of the field it covers.

The experience of the authors is amply reflected in the manner of presentation, and the second portion of the book, concerned with the significance and interpretation of spinal fluid findings, should be of great value to the practitioner.

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*The Cerebrospinal Fluid in Clinical Diagnosis. By J. G. Greenfield, Pathologist to The National Hospital for The Paralysed and Epileptic and E. A. Carmichael, Resident Medical Officer. Pp. 272. 8 illustrations. Cloth. $5.00. Macmillan and Co.

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EDITORIALS

The Respiratory Hormone

STARTING with the work of Haldane and his school widespread interest has in recent years been taken in the nature of the respiratory hormone, that is, in the nature of the chemical changes occurring in the respiratory center which influence the rate and depth of breathing. Since it is known that hyperpnea may be brought about by (*a*) an increase in the CO_2-tension, (*b*) a decrease in the O_2-tension (anoxemia) and (*c*) an increase in P_H of the arterial blood, each of these has in turn been designated as the respiratory hormone, but recent work, especially by Gesell and his collaborators, has shown that no one of them can be the actual respiratory hormone; all three are related to, but none is *the* respiratory hormone. What may this be? Of the three changes the one that is least related to breathing is the P_H of the blood, a fact which has recently been most strikingly demonstrated by Gesell who has devised a method by which it is possible to make continuous observations of the P_H of the arterial blood in a living animal, by

inserting a manganese dioxide *electrode* in a blood vessel. With McGinty he has found that hyperpnea may occur when there is an actual increase in P_H (i.e., decreased acidity) such as following the intravenous injection of solutions of sodium bicarbonate, in anoxemia, or as result of hemorrhage. There is, therefore, no close relationship between hyperpnea and the reaction of the arterial blood. A somewhat closer parallelism exists between hyperpnea and anoxemia, but this does not carry us far in explaining the nature of the hormone, for it is unlikely that a deficiency of O_2 *per se* could act as a stimulus, and it is really only begging the question to state that lack of oxygen increases the sensitivity of the center towards the H-ion; the question remains as to what the actual stimulus may be. Changes in the third factor, the tension of CO_2 of the arterial blood, is perhaps the one which most closely parallels respiratory activity, but even in this case the relationship is not sufficiently pronounced to warrant our considering the arterial CO_2 as the respiratory hormone. For example, although a quantitative increase occurs in breathing when the alveolar CO_2 is raised, as by breathing in CO_2-rich atmospheres, a low arterial CO_2 tension occurs in the hyperpnea due to anoxemia.

Gesell has satisfactorily brought into harmony all these discordant hypotheses by pointing out that *the true respiratory hormone is the H-ion concentration of the respiratory center itself*. This will depend upon several conditions of which the following are the most important: (1) the oxidative processes in the protoplasm of the nerve cells of the center; (2) the ability of the blood circulating through the center to supply it with oxygen and to carry away the products of its metabolism; (3) the exchange of H-ions between the blood and the center; (4) the buffer influence of the protoplasm of cells of the center. With regard to the first of these conditions, it may be supposed that lactic acid will be formed and removed in the nerve cells, just in the same way as in muscle cells. Evidence that this occurs has been obtained by Gesell and McGinty by determinations of the amount of lactic acid which accumulates in brain tissue *in the first few minutes following* death. The results were similar to those obtained in muscle. It is true that it has been impossible to demonstrate, by comparison of the arterial and venous blood of the brain, that the O_2 consumption is a large one, but, considering the large volume flow of blood as a whole, as well as the fact that only relatively few centers are likely to be active at any one time, the relatively low oxygen consumption is not to be wondered at. The well-known great vulnerability of nerve cells to lack of oxygen on the other hand, is evidence that oxidative processes must be taking place constantly in them.

The second factor comes into play partly through changes in the O_2 tension of arterial blood and partly through changes in the volume flow of blood. When this is sluggish, for example, sufficient O_2 will not be furnished the center, however saturated with this gas the arterial blood may be, nor will lactic acid and CO_2 be removed from it. This is well illustrated in the effect of hemorrhage and subsequent transfusion, breathing being excited

by the former and depressed by the latter. It is significant also that P_H of the arterial blood decreases following hemorrhage and increases following transfusion.

The third factor involves several subsidiary ones of which may be mentioned: (*a*) the P_H of the circulating blood; (*b*) the relative rates of diffusion of ions between the blood and the nerve cells. Of course, the higher the P_H of the blood the higher will tend to be the P_H of the center, other things being equal, but, as we have seen, it is possible for considerable differences in this regard to become established within and without the cell wall. Besides the experiments of Jacobs, Gesell, etc., referred to, Gesell and Hertzman have shown, by their method of continuous measurement of P_H, that when CO_2 was administered the acidity of the arterial blood rose considerably more than that of the venous, indicating that CO_2 must have diffused into the tissue cells. On the other hand when bicarbonate solutions were injected the changes in P_H were practically the same in arterial and venous blood, indicating that the cells had failed to absorb the bicarbonate. These facts indicate that carbonic acid will readily penetrate into the cells of the respiratory center with the result that the P_H of the contents becomes raised and breathing stimulated, whereas bicarbonate will fail to pass into them, so that breathing will be relatively unaffected.

The fourth factor is difficult to appraise. It is probable that the buffer influence of the cells is much lower than that of the blood so that changes in P_H will readily occur and it is also to be remembered that when lactic acid is formed it will affect the P_H of the center relatively more than an equivalent quantity of CO_2, because of its lower diffusibility through the cell wall.

—*J. J. R. M.*

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CLINICAL AND EXPERIMENTAL

SOME IMPORTANT PHASES OF THE DIABETIC PROBLEM WITH SPECIAL REFERENCE TO DIAGNOSIS*

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DIABETES mellitus is on the increase in the United States. Joslin[1] estimates that we have a million diabetics at the present time and insists that a campaign for its prevention holds the same promise and importance as preventive measures against tuberculosis, typhoid fever, and other diseases. The approach to the problem of prevention[2] lies along two lines, first, to detect the disease early, and second, to preclude its development in those known to be susceptible to it.

EARLY DIAGNOSIS

The early diagnosis[3, 4, 5] of diabetes in most cases is simpler than the early diagnosis of tuberculosis. It depends primarily on routine urine examinations by the general practitioner in all cases. In questionable cases the urine examinations should be supplemented by a fasting blood-sugar determination. Provided these two tests are made, few early diabetics will be overlooked. It is particularly important that obese individuals, patients with a suggestive hereditary or familial tendency to diabetes, patients convalescing from acute infectious diseases, and cases of chronic cholecystitis should have the foregoing examinations.

Life insurance examinations[6] have served to disclose a great number of silent glycosurias, and the plan of offering free urine examinations to policy

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holders will increase the number of early diabetics discovered. Likewise, specialists, including the dental surgeons, are demanding complete medical examinations before initiating operative procedures, so that an occasional case will be detected in this way.

I wish to direct attention to an important group which I am sure is common, but which offers certain difficulties in the diagnosis. This group is characterized by the presence of a transient glycosuria without a hyperglycemia or any of the usual clinical symptoms of diabetes, and cases of this group are frequently discovered in Life Insurance examinations and other routine medical surveys. The presence of glucose in the urine is found on the first examination, but on a repeated test, a few days later, glucose is absent. The consultant then sees the case, and sugar is again found. The *important problem is, obviously, what is the significance of a transient glycosuria?* The question can only be answered by studying the glucose tolerance,[7, 8, 9, 10] as determined by a blood-sugar curve following a dose of glucose. As previously mentioned, the fasting blood-sugar level is often normal, so that this determination alone affords no help. Interesting information can be obtained, however, *by administering to the patient, on the fasting* stomach, 100 grams of glucose dissolved in 400 c.c. of water to which the juice of an orange or lemon is added, and by plotting the curve of the blood-sugar levels at the half hour intervals up to two hours. In order to determine the fasting blood-sugar level and the presence, or absence, of sugar in the urine, *it is important to collect the urine and the blood previous to the administration of the glucose solution.* Besides the quantitative determinations of the glucose content of the blood at the half hour intervals, qualitative tests for the presence of sugar in the urine are also made.

THE SALIENT POINTS IN THE INTERPRETATION OF THE GLUCOSE TOLERANCE TEST

The individual who possesses the normal mechanism for glucose utilization will not develop a glycosuria on 100 grams of sugar at any period of this test. The amount of cane sugar which can be taken by most adults before sugar appears in the urine is from 200 to 250 grams.

Of greater importance, however, is the height to which the blood-sugar curve rises, the time required for it to reach its maximum height, and the time required for it to return to the normal fasting level. A maximum rise to a point over 0.17 per cent is abnormal, and a delay in return to the fasting level beyond the two hour interval usually indicates an impaired glucose utilization. Of the two criteria, the latter undoubtedly has greater significance. Disturbed utilization is also suggested by a curve which tends to rise to the maximum point slowly, since normally the rise to the maximum occurs promptly within the first half hour.

Unfortunately, the type of curve obtained is not pathognomonic of diabetes in all cases. Extensive studies[11, 12, 13] on the variations of the blood sugar have shown that other conditions than diabetes are at times associated with impaired glucose utilization and likewise abnormal blood-sugar curves. Chronic nephritis with hypertension, essential hypertension, such diseases of

the glands of internal secretion as hyperthyroidism and acromegaly, and gastric carcinoma are among the important well-known diseases that show abnormal curves. Nevertheless, with these conditions excluded, the glucose tolerance test offers evidence with regard to borderline diabetics which cannot be obtained by any other method.

It is my impression that it has not been sufficiently emphasized in literature that the estimation of the fasting blood sugar alone is not of diagnostic value, since in even a severe case of diabetes the fasting blood sugar may be normal, if the patient has been on a diet previous to the test. For this reason the glucose tolerance test has come to have an especial value in the study of the prediabetic, mild, and borderline cases.

A WIDER CONCEPTION OF THE SIGNIFICANCE OF GLYCOSURIA

Glycosuria, in itself, can no longer be regarded as a sign of a single disease. It appears more rational to think of glycosuria as a symptom like jaundice, angina, or headache; the cause of which must be ascertained in each case.

IDENTIFICATION OF A URINARY SUGAR

Any urine which shows a reducing substance with our copper reagents (i.e., Benedict's or Fehling's solutions) must be studied to determine which sugar, or sugars, is present. All reducing substances are not glucose, although glucose is by far the most common urinary sugar. Levulose, lactose, maltose, pentose, glycuronic acid and several other substances reduce copper solutions. It is well to remember, however, that glucose and levulose are the only sugars fermented by yeast. To differentiate glucose and levulose the polariscope is most useful, since glucose is dextrorotatory and levulose is levorotatory. Perhaps the most interesting characteristic of levulose is its failure to produce a hyperglycemia even after a 50 gram dose. It is believed that the absence of any appreciable elevation of the blood sugar after the administration of levulose by mouth may be attributed to the efficiency of the liver in removing this particular sugar from the blood and storing it as glycogen. In cases in which there is an inability on the part of the liver to deal with the ingested levulose, clinical evidence of hepatic disease is usually present; however, tests of this type do not permit of any exact quantitative estimate of the degree of impairment of the liver function.[14]

The presence of a substance which reduces copper solutions and at the same time does not ferment yeast, immediately puts it in the class of the unusual substances. Lactosuria[15, 16] of pregnancy and the lactating state is perhaps the most confusing and frequently encountered urinary sugar besides glucose. It can be differentiated from glucose by Mathews' method,[17] the essential basis of which is the fermentation of urine with a large amount of yeast. The glucose is quickly fermented away and its reducing action destroyed, while lactose remains unaffected in the filtrate. The identification of the other urinary sugars and reducing substances requires the application of special chemical tests which are described in books on clinical diagnostic methods.[18]

THE RENAL THRESHOLD

The term renal threshold has come into common usage since the advent of routine blood-sugar determinations. It means the concentration of the sugar in the blood at which point sugar first appears in the urine. It is now thought that this point is a blood-sugar concentration of about 0.17 per cent; however, this is a variable level not only in different individuals but also probably in the same individual at different times. The renal threshold value has come into practical importance chiefly because of its variations both above and below the normal average level of 0.17 per cent.

RENAL GLYCOSURIA

An interesting familial condition, first described by Klemperer[19] about thirty years ago, emphasized a lowering of the renal threshold so that an anomalous condition came about, in which glycosuria existed with a normal glycemia. This was first called renal diabetes but is now usually referred to as renal glycosuria. Subsequent studies have shown that it is characterized by four cardinal findings; namely, first, specimens of urine and blood collected simultaneously must demonstrate a glycosuria with a normal or depressed glycemia; second, there must be no symptoms of clinical diabetes mellitus present, i.e., loss of weight, polyuria, polydipsia, etc.; third, glycosuria must not parallel the carbohydrate content of the diet, i.e., increase of carbohydrate in the diet should not increase the amount of sugar in the urine; fourth, the patient must not later develop into a true case of diabetes mellitus. This latter stipulation Joslin has always stressed, since he feels that unquestionable instances of renal glycosuria are uncommon and that many of the reported cases have not been followed for a sufficiently long period to be sure that true diabetes has not eventually developed. This attitude of conservatism in the diagnosis of renal glycosuria, I feel, has much to commend it, particularly until more has been learned about the mechanism controlling the renal threshold for glucose and until the ultimate outcome of the established cases of this condition has been followed for a longer time.

ELEVATION OF THE RENAL THRESHOLD

The opposite condition to renal glycosuria with reference to the renal threshold, namely, a hyperglycemia without glycosuria, ocurs frequently in patients who have been treated with insulin. Major and Davis[20] have recently reported a short series of such cases. Advanced renal sclerosis is also occasionally accompanied by this condition. At the present moment there is no accepted explanation for the elevation of the renal threshold in these cases. Allen[21] has considered this point in connection with some of his dog experiments. He is strongly of the opinion that this raising of the threshold is a disadvantage to the animal, since the internal secretion of the pancreas is being overworked whenever the level of the blood sugar is considerably raised. With this fact in mind, it seems rational to try to keep the blood sugar at the normal level at all times in diabetes by diet restriction and proper

insulin dosage, including both the total amount of insulin given and the spacing of the doses.

NONDIABETIC GLYCOSURIAS ASSOCIATED WITH AN ELEVATED BLOOD SUGAR

It is gradually becoming apparent that there are some clearly defined groups of cases which present glycosuria with hyperglycemia, but which are not true instances of diabetes. I shall try to confine this discussion to four such groups.

The first comprises those examples of excessive ingestion of sugar by a normal individual. This is the so-called alimentary glycosuria[22] and is frequently found in young persons following an excessive intake of candy.

The next group of importance we may term the neurogenic[23, 24] type. Occasionally it is found that a student following an examination passes sugar in the urine. This emotional glycosuria is believed to be the result of an overproduction of glucose in the sense that, because of the nervous stimulation of the liver, there is a rapid conversion of glycogen to glucose, with the accumulation of the latter in the blood stream. Glycosuria follows this elevation of the blood sugar.

Other forms of the neurogenic glycosuria of a similar pathogenesis are seen after fracture of the skull, intracranial hemorrhage, and during toxic states of acute infections. The association of unconsciousness and glycosuria has an especial clinical significance, since it suggests at once the diagnosis of diabetic coma.

A third type of nondiabetic glycosuria with hyperglycemia occurs during such asphyxia as is commonly produced by carbon monoxide or the anesthetic drugs and is probably due to difficulty in oxidation.

The fourth group is a large and poorly understood one which is associated with disturbance in the glands of internal secretion. It is accepted by physiologists that several of these glands influence the metabolism of carbohydrates. They recognize that glycosuria is a common feature of hyperthyroidism[25, 26, 27] and of exophthalmic goiter, and that associated with this there is usually marked hyperglycemia. The glucose tolerance test shows an abnormal elevation of the blood sugar and in some instances a delay in the return to the fasting level. The diminished carbohydrate tolerance of hyperthyroidism is, in fact, one of the most constant of the abnormalities of carbohydrate metabolism met with in endocrine diseases. Respiratory experiments, however, demonstrate that there is no decrease in the patient's ability to oxidize glucose but that probably there is a defect in the storage mechanism which fails to handle the excessive mobilization of glucose, a diametrically opposite state than that of diabetes.

The glycosuria of pituitary disturbances[28] is another endocrine manifestation and is, supposedly, caused by a hypersecretion of the posterior lobe of the hypophysis. The importance of the pituitary body in controlling the concentration of sugar in the blood is shown by the fact that it is impossible to produce the characteristic convulsions of insulin hypoglycemia in animals in which the cerebral hemispheres have been removed but the pituitary body

left intact. If the pituitary body is also removed, adequate injections of insulin are followed by the typical hypoglycemic reaction. A considerable proportion of the obese patients show a curve suggestive of a mild degree of impairment of carbohydrate tolerance,[29] and it probably is advisable to regard such cases as potential diabetics; at the same time it must be recognized that in some patients of this type the hyperglycemia and obesity may be the expression of a pituitary disorder, often dating from pregnancy.

Epinephrin administered subcutaneously causes a discharge of glycogen from the liver into the blood stream and is frequently associated with a hyperglycemia and at times with a glycosuria. The hyperglycemia and glycosuria that follow puncture of Claude Bernard's "diabetic center" in the floor of the fourth ventricle are believed to be due to a stimulation of the splanchnics and excessive secretion of epinephrin.

GLYCOSURIA WITH THE HYPERGLYCEMIA OF TRUE PANCREATIC DIABETES

I will now pass to a brief consideration of true pancreatic diabetes with glycosuria and hyperglycemia. In this condition the abnormality lies in the *oxidation of glucose rather than in its mobilization or ingestion.* Diabetes mellitus is due to a disturbance in the function of the Islands of Langerhans of the pancreas, the primary inciting cause of which, in most instances, is still unknown, although infection and degenerative arteriolar processes probably play an important part.

In spite of the fact that this disease is usually comparatively simple to recognize clinically, difficulty in diagnosis is encountered in three groups of cases: (1) Asymptomatic glycosuria; (2) patients complaining of the complications of diabetes only; (3) patients first seen in coma.

THE ASYMPTOMATIC GROUP

The characteristics of this group and the modern method of diagnosis have already been discussed under the heading of early diagnosis. I wish to reemphasize the extreme importance of this type of case and to insist that the physician must be on the alert to the problem presented. Dietetic restriction and insulin therapy are, obviously, contraindicated in nonpancreatic glycosurias. On the other hand, to dismiss an individual casually, with the assurance that his glycosuria is of no significance, may eventually throw him into the class of uncontrolled diabetes. Each case should be studied carefully and an opinion reached on the merits of the findings. Naunyn's teaching, that all glycosurias should be regarded as diabetic until they are proved otherwise, still holds true.

PATIENTS COMPLAINING OF ONE OF THE COMPLICATIONS OF DIABETES AS THEIR PRESENTING SYMPTOM

This group is easy to diagnose provided diabetes is thought of as the underlying cause of an apparently unrelated condition. For example, I recently discovered a well-marked glycosuria in a patient who had been treated by a competent ophthalmologist for months for a neuroretinitis. Apparently the diabetic etiology of this eye condition had never been considered.

Similarly I have recently seen an elderly gentleman who had been suffering for months with a generalized pruritis and an eczematous skin eruption on the dorsum of the feet and around the ankles. His family physician had treated him by the application of local remedies for several months without satisfactory results. Finally the patient consulted a neurologist, who promptly found a large amount of sugar in the urine. Such examples could be multiplied. Several instances of diabetes treated locally by the urologist for sexual impotence have come to my notice. In all instances, the difficulty has been the failure to consider the possibility of the underlying cause for a particular symptom; in other words, the attention has been focussed too closely on the complication, whereas the cause has been overlooked.

PATIENTS FIRST SEEN IN COMA

Unconsciousness in a patient with glycosuria demands use of the physician's utmost diagnostic skill, in order that he may avoid hasty and harmful injection of insulin, to adjust the treatment to both the diabetes and probable complications, and to protect the rights of the patient's family. Coma is the state of unconsciousness which follows diabetic acidosis. Clinically, it is characterized by "air hunger" and, chemically, by an excess in the blood and urine of the end-products of faulty fat metabolism. The onset of coma is usually gradual and is associated with anorexia, headache, abdominal cramps, and vomiting. The precipitating cause is usually an acute infection, a nervous upset, or an unusual exertion, but the real cause is always dietary error. The physical findings show a patient with deep and rapid respiration, dry skin, cold extremities, subnormal temperature, rapid pulse, low blood pressure, soft eyeballs, flushed cheeks, and at times an odor of acetone on the breath.

Joslin[30] has recently emphasized some of the special points of clinical importance in diabetic coma. He points out that the fat diabetic can go into coma, but it takes gross mismanagement to produce it. In his series of 48 coma cases only one exceeded 140 pounds at the time of coma. The leucocytosis frequently found is usually secondary to gastric hemorrhage, provided infection can be excluded. The preliminary examination should be made for the purpose of seeking an endogenous cause for the coma by searching for localized infections, pneumonia, and such intraabdominal complications as a ruptured appendix, kidney complications, or paranephric abscess. The blood pressure is an important determination and often offers a valuable index of the patient's condition and progress. It tends to run comparatively low in most cases, the systolic pressure frequently being found under 100 mm.

The essential laboratory findings vary, but a catheterized specimen of urine will show sugar in moderate amount, whereas the blood sugar is relatively higher and often falls between 0.40 per cent and 0.80 per cent. The urine gives a maximum red-black reaction to ferric chloride for the presence of diacetic acid. If the facilities for the determination of the carbon dioxide combining power of the blood plasma are available, it is usually found below 15 per cent by volume; when above 30 per cent by volume, it betrays either a complication or the previous administration of alkali. Joslin has shown that the human organism is tolerant to an extraordinary degree to extreme alter-

tions in the carbon dioxide content of the blood, and he "wonders whether acidosis and alkalosis ever reach such a stage in the human body as of themselves to cause death. Are not the deaths which we associate with these variations in the blood carbon dioxide really due to other factors in the overwhelming majority of instances? Such factors are intercurrent infections, septicemias, heart failure from exhaustion, a dilated stomach, or lack of body fluids."

Other causes for the clinical picture simulating coma are numerous and include such things as uremia, apoplexy, meningitis, trauma, hypoglycemia due to inanition or overdosage with insulin, narcotic poisoning, and brain tumor.

SUMMARY

To control the increasing prevalence of diabetes in this country it is necessary to detect the early cases and to preclude the development of this disease in those groups of individuals known to be susceptible to it.

Special attention is called to a large group of early or so-called latent cases in which the proper study of the individual furnishes important data for the future management of the patient. In this group the glucose tolerance test supplies significant information not obtainable by any other means. The estimation of the fasting blood sugar alone is not always of diagnostic value, since even in a severe case of diabetes the fasting blood sugar may be normal if the patient has been on a diet previous to the test.

Glycosuria, in itself, can no longer be regarded as a sign of a single disease. It appears more rational to think of glycosuria as a symptom such as jaundice, angina, or headache, the cause of which must be ascertained in each case.

The diagnosis of renal glycosuria should not be arrived at hastily, since unquestionable instances of this condition are uncommon and few cases have been followed for a sufficient period to be certain of the final outcome.

An elevation of the renal threshold, so that hyperglycemia exists without glycosuria, occurs occasionally in diabetics who have been treated with insulin. Present information would indicate that a constant elevation of the threshold is a disadvantage to the organism because of the excessive work continuously thrown upon the internal secretion of the pancreas.

The four important groups of nondiabetic glycosurias associated with an elevated blood sugar content are the alimentary, neurogenic, toxic, and the endocrine.

The endocrine group at the present time represents a large and poorly understood one which is associated with a disturbance in the function of the glands of internal secretion. Perhaps the most outstanding example is the diminished carbohydrate tolerance of hyperthyroidism as shown by the presence of glycosuria and an abnormal blood-sugar curve. The glycosurias accompanying pituitary and adrenal disturbances need further experimental study.

True pancreatic diabetes offers especial difficulty in the diagnosis primarily in the three following groups of cases: (1) Asymptomatic glycosuria; (2) patients complaining of the complications of diabetes only; (3) patients first seen in coma.

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THE SIGNIFICANCE OF CHANGES IN THE COMPOSITION OF THE BLOOD AND URINE AFTER THE INGESTION OF GLUCOSE*

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HARROP and Benedict[1] have observed a fall in the inorganic phosphorus of the blood serum simultaneously with the rise in blood sugar after the ingestion of large doses of glucose. In animals, when the glucose was combined with insulin administration, these authors noted a fall in the inorganic phosphorus of the blood, a rise in the organic phosphorus of the muscles, and a diminished phosphate excretion in the urine. These findings indicate a shift in the mobile phosphate stores of the body during the process of active carbohydrate assimilation. Blatherwick, Bell, and Hill,[2] however, were able to find no consistent change in the inorganic phosphorus of the blood or urine after ingestion of glucose. In some instances an increase was noted, in some a decrease, and in others no change. But after the administration of insulin a marked fall of the inorganic phosphorus of the blood serum and a drop of the phosphorus excretion in the urine were consistently reported. Briggs, Koechig, Doisy, and Weber[3] have shown a decrease in the concentration of glucose, inorganic phosphorus and potassium of the blood of normal animals with a parallel rise in the lactic acid under the influence of insulin. Fiske[4] has studied the influence of carbohydrate ingestion on phosphoric acid excretion in the urine. He states that carbohydrate combustion is accompanied by retention of phosphate, which is later excreted at an exaggerated rate. All of the data recorded in these and similar studies lend additional support to the belief that a combination of hexose with phosphoric acid is an intermediate step in the metabolism of carbohydrate.

With this hypothesis in mind, an attempt was made to get more exact information concerning the metabolism of carbohydrate in glucose tolerance studies in cases of hyperthyroidism, by making simultaneous determinations of the inorganic phosphorous of the blood and urine along with the sugar. The procedure utilized in glucose tolerance studies was that outlined by Killian.[5] The adopted method of handling these cases is as follows: after a fourteen or fifteen hour fast the patients are received in the respiration laboratory for a determination of the basal metabolism.† Thence they come to the chemical laboratory where they receive a standard breakfast, consisting of one egg (boiled), one cup of coffee containing two teaspoonfuls of cream (40 per cent) and two slices of white bread. This meal contains 11.0 grams of fat, 13.7 grams of protein and 37.0 grams of carbohydrate, repre-

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†The basal metabolic rates were determined by Dr. Bailey and to him I am indebted for the privilege of using these data.

senting a total value of 311.7 calories. This breakfast is sufficient to break the fast; the relief of which we find essential for the comfort of the patient. It also makes it possible to study the metabolism of all cases with a definite relation to the time of food intake. Two hours after the breakfast, the bladder is emptied by voluntary voiding, and the patient takes, by mouth, 100 c.c. of water. At the end of one hour after taking the water, a specimen of blood is obtained and a specimen of urine. These two specimens are the controls and represent the composition of the blood and urine three hours after the standard meal. Thereupon glucose is given by mouth, 1.75 grams per kilogram of body weight and in a 50 per cent aqueous solution. During this time the patient is maintained at rest. It has been found that cerelose is a very satisfactory sugar for carbohydrate tolerance tests. Following the ingestion of glucose, specimens of blood and urine are obtained at hourly intervals for two or three hours. The Folin-Wu[6] method was employed for the blood-sugar determinations, and Benedict and Osterberg's[7] method for the urine sugar. Where the sugar of the urine exceeded 0.5 per cent, check determinations were made by the Benedict volumetric method. For the phosphorus Benedict's technic[8] was followed. In all cases care was taken to prevent hemolysis.

In Table I are recorded the data obtained on 9 cases of hyperthyroidism. It will be seen that, although in no instance does the blood obtained at the end of the control hour show a hyperglycemia, the sugar output in the urine during this period is abnormally high. Killian has reported a normal excretion of about 30 mg. for the control hour. The ingestion of the glucose produces, in all but one case, a hyperglycemia persisting for two hours. In Cases 1 and 2 the urine sugar rises markedly in response to changes in the blood sugar, but in the other instances the effect upon the urine sugar is less marked, and in Subject No. 5 the urine sugar remains unchanged. It is not possible to demonstrate any parallelism between the elevation of the basal metabolic rate and the character of the blood-sugar curve after the glucose. This is to be expected. The basal metabolism is a measure of the energy produced in oxidation reactions within the body in a postabsorptive state. The conversion of glucose, however, into glycogen through the intermediate stage of hexose phosphate and lactic acid evolves only 5 per cent of the total caloric value of the glucose. There is noted no consistent change in the inorganic phosphorus of the blood. Cases 7 and 8 show slight increases, the others a slight decrease. The maximum decrease is usually found after the blood sugar has begun its return to normal, but this decrease in the blood inorganic phosphorus is insignificant when compared with the changes in the blood sugar. With the exception of Cases 8 and 9, in all instances the ingested glucose produced a marked increase in the urine phosphorus, which, however, bore no relation to the amount of sugar excreted. In Patients 8 and 9 the phosphorus was diminished in the urine.

Similar observations have been made on 13 cases representing normal and miscellaneous pathologic conditions, without, however, any detectable

TABLE I

CHANGES IN SUGAR AND INORGANIC PHOSPHORUS OF BLOOD AND URINE AFTER INGESTION OF GLUCOSE IN HYPERTHYROIDISM

CASE	AGE	SEX	TEST HOUR	BLOOD SUGAR MG.	BLOOD INORGANIC PHOSPHORUS MG.	URINE SUGAR MG.	URINE INORGANIC PHOSPHORUS MG.	BASAL METABOLIC RATE PER CENT	REMARKS
1. A. W.	40	M	Control	106	5.7	76	10.1	+108	Toxic exophthalmic goiter
			1st	246	5.6	744	17.6		
			2nd	224	5.4	1110	58.3		
			3rd	91	5.8	2642	45.3		
2. L. P.	19	F	Control	93	4.3	312	20.0	+ 95	Exophthalmic goiter
			1st	205	3.8	2627	18.6		
			2nd	150	3.7	3814	22.1		
			3rd	75	4.4	316	28.1		
3. L. B.	32	M	Control	95	4.8	74	56.2	+ 72	Exophthalmic goiter
			1st	192	4.3	786	85.2		
			2nd	150	3.7	363	80.0		
			3rd	69	4.3	105	46.8		
4. J. P.	38	F	Control	78	3.7	69	19.4	+ 51	Exophthalmic goiter
			1st	211	3.6	145	35.0		
			2nd	148	3.4	462	45.0		
			3rd	75	3.9	74	26.6		
5. M. K.	37	F	Control	102	4.0	32	31.2	+ 12	Small goiter with hyperthyroidism
			1st	205	4.1	28	41.3		
			2nd	156	3.9	31	38.8		
			3rd	115	4.1	28	29.7		
6. A. L.	45	F	Control	105	5.5	64	34.3	+ 71	Exophthalmic goiter
			1st	184	4.9	100	47.0		
			2nd	174	5.4	—	—		
7. E. L.	28	F	Control	115	4.5	19	14.4	+ 56	Exophthalmic goiter
			1st	130	5.3	123	66.2		
			2nd	100	5.3	70	74.9		
8. W. L.	23	M	Control	114	3.8	66	29.6	+ 33	Hyperthyroidism
			1st	227	4.2	81	24.6		
			2nd	203	3.2	1770	22.0		
9. B. W.	20	F	Control	98	4.6	72	39.6	+ 32	Exophthalmic goiter
			1st	275	4.5	510	18.0		
			2nd	204	4.3	505	14.0		

change in the thyroid function. The results obtained are recorded in Table II. In this group the basal metabolic rates ranged from 11 per cent below to 14 per cent above the average normal. Four subjects were normals, and they present changes in the sugar of the blood and urine after the glucose, within the limits considered by Killian as typical of the normal. The remaining cases, however, show a mildly lowered tolerance for carbohydrate, but there is no evident relationship between the blood- and urine-sugar curves and the variations in heat production as measured by the basal metabolism. The effect produced upon the inorganic phosphorus of the blood and the urine of the normals by the glucose is analogous in all respects to that observed in cases showing a moderate decrease in carbohydrate tolerance, and in cases of hyperthyroidism presenting a much greater lowering of glucose assimilation. In one instance the blood phosphorus rose from 5.0 to 5.6 in two hours, paralleled by a decrease in the urinary phosphorus from 29 to

TABLE II

CHANGES IN SUGAR AND INORGANIC PHOSPHORUS OF BLOOD AND URINE AFTER INGESTION OF GLUCOSE IN NONHYPERTHYROIDISM

CASE	AGE	SEX	TEST HOUR	BLOOD SUGAR MG.	BLOOD INORGANIC PHOSPHORUS MG.	URINE SUGAR MG.	URINE INORGANIC PHOSPHORUS MG.	BASAL METABOLIC RATE PER CENT	REMARKS
1. F. C.	16	F	Control	84	5.7	22	17.6	+ 1	Normal
			1st	133	5.2	31	30.8		
			2nd	100	5.6	14	22.8		
2. G. D.	12	M	Control	106	5.0	34	29.0	−11	Normal
			1st	144	5.2	17	12.5		
			2nd	119	5.6	16	19.6		
3. B. F.	9	M	Control	110	5.2	21	28.5	+ 2	Normal
			1st	144	5.2	18	33.0		
			2nd	138	5.0	23	25.0		
4. H. N.	13	M	Control	80	5.0	29	19.8	− 2	
			1st	214	4.9	33	28.5		
			2nd	147	5.0	53	51.2		
5. J. B.	15	M	Control	110	4.7	50	23.4	+14	Speech defect
			1st	161	4.7	59	25.3		
			2nd	165	4.5	44	25.1		
6. J. V.	17	M	Control	100	5.1	38	17.1	+ 6	Mental defective
			1st	200	4.9	83	32.7		
			2nd	163	4.7	157	37.8		
7. G. L.	20	M	Control	79	4.5	73	29.9	+12	Normal
			1st	152	4.3	—	—		
			2nd	107	4.6	198	59.7		
8. C. L.	9	M	Control	89	5.3	50	42.0	− 3	Emotional instability
			1st	195	5.1	20	31.4		
			2nd	144	5.2	32	42.2		
9. A. G.	29	F	Control	106	4.0	77	8.0	+ 1	Obesity
			1st	195	3.9	67	21.3		
			2nd	147	4.0	61	20.7		
10. B. K.	23	F	Control	87	4.7	41	12.8	+ 1	Epilepsy
			1st	208	4.1	708	30.5		
			2nd	130	4.1	412	15.0		
11. E. O.	20	M	Control	99	3.4	64	28.3	− 5	Psychoneurosis
			1st	176	3.1	57	35.2		
			2nd	144	3.6	54	37.5		
12. S. P.	25	M	Control	86	4.4	30	26.8	− 9	
			1st	202	3.6	37	40.3		
			2nd	147	3.7	46	40.7		
13. H. M.	17	M	Control	116	5.0	28	20.0	+13	Psychoneurosis
			1st	125	5.0	39	42.0		
			2nd	135	4.8	49	49.2		
			3rd	86	4.7	32	38.4		

19.6 mg. per hour. In all other instances a fall in the inorganic phosphorus of the blood is accompanied by an increase in the urine, and this rise in the urinary phosphorus steadily increases to reach its maximum at the end of the first hour after the glucose and then declines. A comparison of the data in Tables I and II demonstrates that the changes in the inorganic phosphorus of the blood or of the urine bear no quantitative relation to the rate at which the glucose is removed from the blood. Harrop and Benedict[1] observed the

Table III

Changes in Sugar and Chloride of Blood and Urine after Ingestion of Glucose in Hyperthyroidism

Case	Age	Sex	Test Hour	Blood Sugar Mg.	Blood Cl as NaCl Mg.	Urine Sugar Mg.	Urine Cl as NaCl Mg.	Basal Metabolic Rate Per Cent	Remarks
1. E. P.	19	F	Control	93	478	312	620	+95	Exophthalmic goiter
			1st	205	462	2627	460		
			2nd	150	486	3814	560		
			3rd	75	478	316	170		
2. J. P.	38	F	Control	78	495	69	440	+51	Exophthalmic goiter
			1st	211	495	145	280		
			2nd	148	495	462	240		
			3rd	75	495	74	620		
3. E. W.	29	F	Control	90	554	51	828	+46	Adenomatous goiter with hyperthyroidism
			1st	107	548	52	348		
			2nd	123	511	84	402		
			3rd	124	508	60	187		
4. L. B.	32	M	Control	95	500	74	423	+72	Exophthalmic goiter
			1st	192	463	786	440		
			2nd	150	500	363	352		
			3rd	69	488	105	215		
5. G. G.	33	F	Control	83	500	92	902	+60	Exophthalmic goiter
			1st	258	495	801	192		
			2nd	214	495	750	280		
			3rd	68	495	56	200		
6. M. G.	32	F	Control	125	503	44	392	+38	Hyperthyroidism
			1st	238	503	238	200		
			2nd	187	511	350	273		
7. F. L.	50	F	Control	95	511	85	600	+11	Hyperthyroidism
			1st	246	511	333	110		
			2nd	242	511	1125	207		
8. M. P.	17	F	Control	130	525	60	608	− 4	Hyperthyroidism
			1st	170	513	157	203		
			2nd	79	513	273	270		

maximum fall in blood phosphorus about two hours after the glucose; this is about the same as the decrease seen in the cases reported above. This drop in blood inorganic phosphorus is minute in comparison with the sugar assimilated. Since it is accompanied, moreover, by a simultaneous rise in the urine phosphorus, it does not appear plausible to explain the decrease in the blood as due to demands made upon the blood phosphorus for the formation of hexose phosphate. It is seen from the tables that the maximum rise in the urinary phosphorus appears after the blood sugar has begun its return to normal. A possible explanation of this increased output of phosphorus suggests itself. Phosphorus may be drawn from the tissues through the medium of the blood for the formation of the hexose phosphate. With the storage of the hexose as glycogen, the phosphorus is released and excreted in the urine as inorganic phosphorus.

It seemed possible that this depressing effect might be extended to some of the other inorganic constituents of the blood in addition to the phosphates. Changes in the chlorides of the blood and urine produced by glucose taken by mouth have been studied in 8 subjects with hyperthyroidism and in 9 without hyperthyroidism. The results obtained are presented in Tables III

TABLE IV

CHANGES IN SUGAR AND CHLORIDE OF BLOOD AND URINE AFTER INGESTION OF GLUCOSE IN NONHYPERTHYROIDISM

CASE	AGE	SEX	TEST HOUR	BLOOD SUGAR MG.	BLOOD Cl AS NaCl MG.	URINE SUGAR MG.	URINE Cl AS NaCl MG.	BASAL METABOLIC RATE PER CENT	REMARKS
1. D. C.	20	M	Control	82	475	40	1250	+ 6	
			1st	86	475	51	1150		
			2nd	84	475	58	1290		
			3rd	64	475	42	990		
2. F. C.	30	M	Control	77	500	216	1316	+18	Mitral stenosis
			1st	130	500	48	150		
			2nd	126	500	105	209		
			3rd	106	500	51	137		
3. K. P.	21	M	Control	134	513	545	32	–35	Nephrosis (Cholesterol = 0.286)
			1st	147	525	823	34		
			2nd	135	500	895	40		
4. A. M.	31	F	Control	110	528	63	832	+ 2	Obesity (Thyroid therapy for eight months)
			1st	148	512	38	410		
			2nd	92	495	52	519		
5. L. D.	55	F	Control	99	511	48	884	+ 3	Hysteria
			1st	205	503	49	557		
			2nd	150	495	44	441		
6. E. H.	15	M	Control	93	488	54	813	– 1	Epilepsy
			1st	166	475	53	312		
			2nd	165	471	32	62		
7. G. W.	26	F	Control	88	495	58	760	+15	Encephalitis Lethargica
			1st	140	478	67	756		
			2nd	120	495	55	720		
8. H. M.	17	M	Control	116	513	28	730	+13	Psychoneurosis
			1st	125	486	39	274		
			2nd	135	488	49	456		
			3rd	86	500	32	322		

and IV. Whitehorn's method was utilized for the determination of the chlorides of the whole blood, and the Volhard-Harvey procedure for the urine chlorides. In a few instances the blood chlorides remained unchanged after the glucose administration (Nos. 2 and 7 of Table III, and 1 and 2 of Table IV) and in but one case was there an increase (No. 6 of Table III). The remaining cases showed a decrease in the blood chlorides with a return to the control concentration in about three hours. The minimum figure obtained for the chlorides does not always correspond in time with the maximum blood sugar. Where the chlorides return to their control concentration, this return lags behind the drop in the blood sugar. There is, moreover, no apparent quantitative relation between the drop in the blood chlorides and the height of the blood-sugar curve. In Case 5 (Table III) the rise in blood sugar is 175 mg. per 100 c.c., but the drop in the chlorides is but 5 mg. per 100 c.c. On the other hand, in No. 3 (Table III) the blood chlorides decrease 46 mg., but the sugar rises only 34 mg. When, however, the rate of assimilation of the glucose is gauged by a consideration of the blood-sugar curve in association with the urine-sugar curve, there appears to be some dependence of the fall of the blood chlorides upon the amount of the glucose utilized. An accurate estimate of the glucose assimilated cannot be made

TABLE VI

CHANGES IN SUGAR AND NITROGEN OF BLOOD AND URINE AFTER INGESTION OF GLUCOSE

CASE	AGE	SEX	TEST HOUR	BLOOD SUGAR MG. PER 100 C.C.	BLOOD N. P. N. MG. PER 100 C.C.	URINE SUGAR MG. PER HOUR	URINE TOTAL N. MG. PER HOUR	URINE VOLS.	BASAL METABOLIC RATE PER CENT	REMARKS
1. M. B.	26	F	Control	115	30.0	32	263	21	+6	Hyperthyroidism
			1st	288	30.0	1700	541	170		
			2nd	242	26.8	720	291	48		
2. L. M.	27	F	Control	100	28.9	20	550	204	+4	Hyperthyroidism
			1st	227	32.3	320	436	64		(very excitable)
			2nd	103	32.1	99	491	74		
3. F. F.	15	F	Control	91	24.0	80	650	260	−2	(Headaches)
			1st	205	30.0	144	500	75		
			2nd	182	24.0	1292	636	140		
4. M. W.	17	F	Control	83	30.0	43	357	50	+1	Epilepsy
			1st	174	29.0	75	392	40		
			2nd	106	28.0	97	566	170		
5. M. N.	40	F	Control	98	21.1	40	157	33	−9	Arteriosclerosis
			1st	98	21.0	35	206	43		Gastric neoplasm
			2nd	135	21.0	37	210	32		
			3rd	108	21.0	20	97	17		

possible that a study of the changes in the lactic acid content of the blood, following the ingestion of glucose along with the blood- and urine-sugar curves, might give valuable information concerning the rate of glycogenesis. On this point, however, the opinions of some investigators are at variance. Mendel, Engel, and Goldscheider[10] reported no changes in the lactic acid of the blood in two individuals after taking 50 grams of glucose by mouth. Collago and Lewicki[11] found in the blood of normal individuals and diabetics similar amounts of lactic acid; they also found a rise in the blood lactic acid in both groups of cases after the ingestion of sucrose. Nishimura[12] has found an increase in the lactic acid of the blood after taking 1.75 grams of glucose per kilogram of body weight.

In a group of 10 cases, including 6 hyperthyroids, 2 psychoneuroses, and 2 normals, the variations in the lactic acid of the blood have been followed with the blood and urine sugar after the taking of glucose. During the entire period of observation the subjects were maintained at rest, lying upon a couch.

Clausen's method[13] was employed for the lactic acid determinations. The blood was drawn without stasis and analyzed immediately. Under such conditions Nishimura has noted the lactic acid content of normal bloods within the limits of 11.7 to 18.0 with an average of 15 mg. per 100 c.c. It will be seen in Table VII that the cases of hyperthyroidism show a slightly higher figure for the lactic acid in the control blood, ranging from 13.7 to 30.6 mg., the remaining cases present figures from 10.5 to 16.9 mg. per 100 c.c. Following the intake of the glucose, all cases show a rise in the lactic acid, paralleling the hyperglycemia. Cases 3, 5, 6, and 7 show a lowered tolerance for carbohydrate. The glucose in these subjects produces a hyperglycemia persisting for more than two hours, accompanied by a markedly increased output of sugar in the urine. In these instances the average maximum rise in the

TABLE VII

Changes in Sugar and Lactic Acid of Blood after Ingestion of Glucose

CASE	AGE	SEX	TEST HOUR	BLOOD SUGAR MG.	BLOOD LACTIC ACID MG.	URINE SUGAR MG.	BASAL METABOLIC RATE PER CENT	REMARKS
1. H. R.		M	Control	98	10.5			
			1st	134	11.7			
			2nd	119	36.9			
2. H. K.	30	M	Control	97	11.7			
			1st	179	17.5			
			2nd	122	15.7			
3. E. P.	19	F	Control	93	17.8	312	+95	Exophthalmic goiter
			1st	205	23.4	2627		
			2nd	150	22.5	3814		
			3rd	75	11.3	316		
4. E. W.	29	F	Control	90	16.2	51	+46	*Adenomatous goiter with hyperthyroidism*
			1st	107	23.8	52		
			2nd	123	27.4	84		
			3rd	124	18.4	60		
5. L. B.	32	M	Control	95	20.8	74	+72	Exophthalmic goiter
			1st	192	24.3	786		
			2nd	150	22.9	363		
			3rd	69	19.8	105		
6. M. G.	32	F	Control	125	30.6	44	+36	Hyperthyroidism
			1st	238	34.2	238		
			2nd	187	32.8	350		
7. A. P.	32	F	Control	100	17.1	63	+10	Hyperthyroidism
			1st	300	20.7	1160		
			2nd	220	18.0	1482		
8. F. K.	14	M	Control	105	13.7	163	+22	Hyperthyroidism
			1st	183	15.9	53		
			2nd	118	14.4	116		
9. F. S.	26	M	Control	99	16.9	63	+ 4	Dementia precox
			1st	183	20.3	3203		
			2nd	79	18.0	121		
10. P. M.	23	M	Control	96	13.9	35	+15	Neurasthenia
			1st	126	15.3	52		
			2nd	132	21.6	29		

lactic acid of the blood after the glucose is from 3.5 to 4.7 mg., but in the remaining cases with fairly normal tolerance for carbohydrate, this rise runs from 5.8 to 26.4 mg. In these cases studied it appears that individuals with lowered tolerance for carbohydrate present less change in the lactic acid of the blood after glucose than cases with normal or increased tolerance. The data reported in Table VII do not warrant general conclusions, but an intensive study of this phase of the problem is under way at present, and the results will be presented in the near future.

SUMMARY

The ingestion by mouth of 1.75 grams of glucose per kilogram of body weight in 50 per cent solution, three hours after a standard meal, produced in the majority of subjects a slight drop in the inorganic phosphorus of the blood with a markedly increased output in the urine. In a few instances the blood phosphorus was increased. In but 3 of the 22 subjects studied was

the inorganic phosphorus of the urine diminished. These changes in the blood and urine phosphorus after glucose were similar for hyperthyroid and nonhyperthyroid subjects. The increased output of urine phosphorus may be due to the release of the phosphate combined as hexose phosphate when the hexose is converted into glycogen.

Changes in blood and urine chlorides after glucose were studied in 16 individuals. In 4, the blood chlorides remained unchanged, in 2 an increase and in 10 a decrease were found. In but one individual was an increased urine chloride noted; the remainder showed a strikingly diminished excretion of chlorides. Changes in the blood chlorides cannot be due to a dilution of the blood. The intake of the glucose produced no appreciable change in the hemoglobin content of the blood in 7 cases reported. A shift of the chlorides from the blood to other tissues appears to be the only tenable explanation of the changes noted.

Following the administration of the glucose in some instances a fall of the nonprotein nitrogen of the blood was accompanied by an increased output of urine nitrogen. In other cases the blood nonprotein nitrogen rose with a fall in the urine nitrogen.

The ingestion of glucose produced a rise in the lactic acid of the blood. This rise was greater in individuals with normal tolerance for carbohydrate than in those with a diminished tolerance.

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URIC ACID STUDIES*

I. Comparison of the Direct and the Isolation Methods of Uric Acid Determination in Blood Filtrates and a Modification of Folin's Method

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THE reaction of uric acid with phosphomolybdic reagents, as well as with phosphotungstic reagents, which results in the formation of a blue color, described by Frabot and by Riegeler,[1] was adapted by Folin and Denis to the determination of uric acid in the blood and other body fluids in 1913,[2] and since then many modifications of the reagent have been proposed. The nature of the compound to which the blue coloration is due is still unknown. The reagents are not specific for uric acid. It is known that the characteristic blue color may also be obtained by the action of the reagents on phenol, polyhydroxi-phenols, certain amino-acids and adrenalin, all of which substances may be present in the protein-free filtrate. This applies equally to Folin's phosphotungstic acid reagent[3] and to Benedict's arsenophosphotungstic acid reagent.[4] Notwithstanding this fact the so-called direct method, which omits any attempt at the separation of the uric acid from these substances, is often employed. The direct method of determination has certain advantages. It shortens the procedure, and the use of sodium carbonate to bring about the necessary alkalinity following the solution of the precipitated urate as uric acid is unnecessary. Furthermore a possible loss of the uric acid present, due to incomplete precipitation, is precluded.

The main difficulty encountered in the present methods is due to the precipitation of a hard, white, crystalline substance, which often occurs before colorimetric readings are obtained. This difficulty is not overcome by the omission of the preliminary isolation, but apparently the frequency with which precipitation occurs is increased, probably because of the greater concentration of tungstates and sulphates in the reaction mixture. If the precipitation occurs shortly after the reagent has been added, it may be removed by centrifugation without loss in color of the supernatant fluid, provided the precipitation was complete before centrifuging. It may happen that after spinning and obtaining a clear, supernatant fluid, the process of precipitation is still continued. A second separation consumes too much time, and the color is likely to fade before readings can be obtained. The same difficulty is encountered when the precipitation occurs just before the solutions are ready for reading. The phosphotungstic salts present are in a supersaturated solution, and the slightest dust or solid particle of any kind will

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initiate precipitation. Since many of the solutions employed, such as the cyanide, the carbonate, as well as the reagent, attack glass, the presence of minute solid particles can only be avoided by keeping them in paraffined containers and by frequent filtering. While the addition of a small amount of lithium sulphate as advocated by Folin,[3] as well as strict observance of the precautions mentioned above, will reduce the frequency with which precipitation occurs, the danger is not avoided. The modification of the method introduced in our experiments and reported in this communication increases the stability of the final solution, so that precipitation practically never occurs, although the precautions as to paraffined containers and frequent filtering are not observed. Furthermore the color developed is more intense and is stable for twenty-four hours.

It has been stated that precipitation of uric acid in the protein-free filtrate by silver lactate in lactic acid solution accomplishes the seemingly impossible, since silver lactate does not react to form insoluble silver urate with uric acid present in the concentrations such as maintain in the blood filtrate, from aqueous solution.[4] The presence of other radicles which form highly insoluble silver salts aids in the precipitation of the silver urate. We have tested the precipitation capacity of the silver lactate reagent on known solutions of uric acid in sulphuric-tungstic acid solution of concentration such as prevails in the blood filtrate and using our modification of Folin's method obtained a recovery varying between 95 per cent and 100 per cent. The average of eight experiments was 98 per cent. Practically the same results were obtained when sodium chloride in N/10 HCl was used as the solvent for the precipitated urate, the recovery varying between 92 per cent and 100 per cent. By using basic zinc carbonate for the preliminary isolation we were unable to recover appreciable amounts of uric acid present in the tungstic acid solution. Our modification of Folin's uric acid method was also tested by running comparative analyses on blood filtrates. The results varied only within the experimental error of the method and proved that the methods are comparable.

Silver lactate in common with other silver salts has a tendency to decompose. Many of the so-called C. P. preparations have a distinct grayish tinge. If this is pronounced, and if there is considerable insoluble material present when making the 5 per cent solution in 5 per cent lactic acid, it is best to determine the silver content of the filtered solution. Since the solution also has a distinct tendency to reduction it must be kept in small amber glass bottles, or the bottles may be painted on the outside with optical black. The addition of NaOH, to partially neutralize the lactic acid, as advocated by Folin,[3] should be avoided, since it increases the tendency to reduction. The presence of reduced silver must be guarded against in the determinations, as the reduced silver forms a double salt with the NaCN which also gives a blue color with the reagent. This source of error has been pointed out by Morris and McLeod,[5] who for this reason prefer basic zinc carbonate as the isolating agent. If during the analysis, however, the reaction mixtures are kept from the direct light and not allowed to stand too long between centrifuging, there

is no danger of formation of free silver. This is readily accomplished by keeping the centrifuge tubes in which the reaction is carried out in the metal containers and allowing them to remain in the closed centrifuge for the five minutes which ought to elapse *before the first spinning.* Our experiments with uric acid added to the blood filtrates, as well as with uric acid solutions in sulpho-tungstic acid, point to the silver lactate as the more efficient precipitant. When the uric acid was added either to whole blood or to plasma prior to the precipitation of the proteins, the recovery of the added uric acid lay between 53 to 72 per cent. That this loss is due to adsorption of the uric acid on the precipitated proteins is borne out by the fact that the loss in concentration was the same whether the determinations were made on the

TABLE I

RECOVERY OF URIC ACID ADDED TO WHOLE BLOOD

CONC. OF URIC ACID BEFORE ADDITION MG. %		ADDED URIC ACID IN MG. %	DETERMINED CONC. OF URIC ACID MG. %		URIC ACID RECOVERED IN PER CENT OF AMOUNT ADDED		% URIC ACID RECOVERED. CORRECTED FOR DRY VOLUME	
Direct	Isolated		Direct	Isolated	Direct	Isolated	Direct	Isolated
4.3	2.8	2.5	6.1	4.5	72%	68%	48%	46%
—	2.5	4.0	—	5.7	—	80%	—	56%
4.8	3.2	3.0	7.0	5.5	73%	77%	51%	54%
2.2	1.8	6.0	6.7	6.5	75%	78%	56%	59%
6.0	4.0	1.5	7.2	5.1	80%	73%	56%	52%
4.5	2.7	5.0	8.5	6.7	80%	80%	53%	53%
					Average recovery		53%	53%

TABLE II

RECOVERY OF URIC ACID ADDED TO BLOOD PLASMA

CONC. OF URIC ACID BEFORE ADDITION MG. %		ADDED URIC ACID IN MG. %	DETERMINED CONC. OF URIC ACID MG. %		URIC ACID RECOVERED IN PER CENT OF AMOUNT ADDED		% URIC ACID RECOVERED. CORRECTED FOR DRY VOLUME	
Direct	Isolated		Direct	Isolated	Direct	Isolated	Direct	Isolated
4.5	2.6	2.5	6.3	4.5	72%	76%	60%	63%
5.00	3.0	3.0	7.5	5.4	83%	80%	69%	67%
2.2	1.5	2.0	4.1	3.5	95%	100%	79%	85%
6.0	4.0	4.0	9.1	7.0	77%	75%	64%	62%
2.5	2.0	2.0	4.5	4.0	100%	100%	83%	83%
					Average recovery		71%	72%

filtrate directly or by preliminary isolation. (Tables I and II.) In plasma the concentration of the adsorbant (the precipitated proteins) is far less than in whole blood, and one would therefore expect a better recovery. The amount of uric acid recovered when it is added to plasma as against that when the uric acid is added to whole blood is far greater than the analyses show. The last two columns on Tables I and II show the corrections for the dry volume, i.e., the volume occupied by the precipitated proteins. As has been pointed out,[6] this error is in direct proportion to the dry volume. Twenty c.c. of the protein-free filtrate obtained from whole blood with an average cell volume of 45 per cent will contain the soluble substances present in approximately 3 c.c. of whole blood, whereas the same amount of filtrate obtained from the plasma of the same blood will contain the soluble sub-

stances present in approximately 2.4 c.c. of plasma. That this error is partially compensated for by the adsorption of the soluble substances present in the filtrate on the precipitate must be conceded, but the extent of adsorption is indeterminate. Correcting for the dry volume the average recovery of the uric acid added to whole blood was 53 per cent, while for the uric acid added to plasma it was 72 per cent.

The results of comparative uric acid analyses, using Folin's as well as Benedict's reagents and carrying out the determinations on the filtrate direct as well as by preliminary isolation, are given in Table III. The results point to the desirability of employing a preliminary isolation method. The analyses given are representative of the results obtained in various pathologic conditions. The difference in results between the two methods (direct and preliminary isolation) while of varying degree is constantly present. Experiments on a small group of "normals" confirmed the findings of Bulmer, Eagles, and Hunter[7] of the existence of a distinctive difference between the two methods. The analyses carried out on the filtrate direct are invariably higher, irrespective of the reagent used. The differences vary from 12 per cent to 120 per cent.

The method used for the determination of the uric acid in the filtrate obtained by Folin's precipitation method was as follows:

Ten, fifteen, or twenty cubic centimeters of the filtrate are pipetted into a clean and dry 50 c.c. pyrex centrifuge tube with constricted end. Five cubic centimeters of the silver lactate reagent (5 per cent silver lactate in 5 per cent lactic acid) are added. The mixture is allowed to stand for five minutes *in the dark* and next centrifuged for five to seven minutes at top speed (2,500 R.P.M.). After carefully decanting the supernatant fluid, 3 c.c. of 10 per cent lithium chloride solution are added, and the mixture is stirred with a very fine glass rod. Eight to ten c.c. of distilled water are next added, the mixture well stirred and the rod finally washed down with about 2 c.c. of water. The tube is centrifuged *immediately* for eight to ten minutes at top speed and the clear supernatant fluid transferred to a 25 c.c. volumetric flask. From a burette 2 c.c. of recently filtered 15 per cent NaCN solution are added. One c.c. of the reagent is now added with shaking and the color allowed to develop for twenty to thirty minutes. The solutions may be made to mark immediately after adding the reagent or just before reading in the colorimeter.

Simultaneously the standard is set up in a 50 c.c. flask, using 1 or 2 c.c. of a standard uric acid solution, containing 0.1 mg. uric acid per c.c., in Benedict's and Hitchcock's phosphate solution.[8] Water to about 30 c.c. is added, next 6 c.c. of 10 per cent lithium chloride solution, 4 c.c. of the NaCN solution and 2 c.c. of the reagent.

The standard uric acid solution in phosphate solution, containing 100 mg. per liter, will keep indefinitely if certain precautions are observed. The standard must be kept in small amber glass containers, or containers painted on the outside with optical black, of approximately 200 c.c. capacity. Prior to use the containers are set up with cleaning fluid overnight, washed with

tap water followed by a thorough rinsing with distilled water, and next steamed for fifteen to twenty minutes. The apparatus described by Findlay for the steaming of conductivity vessels is simple and well adapted to this purpose.[9] The bottles after filling and adding 5 c.c. of redistilled toluol are corked, paraffined, and kept in the ice box. In this way the standard of the bottle in use will be exhausted within a short enough time to eliminate all danger of decomposition due to exposure to the atmosphere. We have found that uric acid solution prepared and kept in this manner will not lose in concentration even though kept for a period of eighteen months. Needless to say the uric acid must be carefully recrystallized before use and the phosphate solution boiled and filtered.

Upon the addition of silver lactate to the blood filtrate, silver chloride, silver tungstate, silver phosphates, as well as silver urate, are precipitated. The use of lithium chloride as solvent for the silver urate in this mixture, as well as a precipitant for the excess silver present, has distinct advantages over the use of NaCl in dilute HCl. Lithium urate is far more soluble than uric acid, and none of the other silver salts present are as readily dissociated by the lithium chloride as by the N/10 HCl. The solution obtained is practically neutral, and the use of the carbonate or of the cyanide in too large a concentration in order to obtain the alkalinity necessary for the final reaction is also avoided. The use of the carbonate, as well as of the cyanide, in large concentration increases the tendency to precipitation. The use of the cyanide in greater concentration than employed in this method is also contraindicated for the reason that the cyanide itself gives a color with the Folin or the Benedict reagents.

Two uric acid standards were set up, using identical quantities with the exception of the cyanide which was doubled in one of the solutions. It was found that using the Folin reagent and doubling the cyanide causes an error in the final reading of 48 per cent, while with the Benedict reagent the error was 25 per cent.

The method has also been adapted for the determination of uric acid in the urine.

Five c.c. of albumin-free urine are pipetted into a 25 c.c. volumetric flask; water to approximately 20 c.c. is added, and the solution rendered neutral to litmus by the addition of 30 per cent NaOH or 25 per cent acetic acid, drop by drop. One to two drops generally suffice. The solution is then made to volume. If the urine contains a large amount of phosphates, they are first dissolved by the addition of a few drops of acetic acid. Urines containing deposits of urates are warmed, diluted one to one with distilled water to prevent redeposit, and 10 c.c. of the cooled mixture pipetted into the 25 c.c. flask. The albumin, if any, is removed from the slightly acidified urine by coagulation and subsequent centrifugation, care being taken to avoid loss by evaporation. Two c c. of the diluted urine (equivalent to 0.4 c.c. of urine) are employed in the estimation. Five c c. of silver lactate reagent are added to the 2 c c. of the dilute urine in a 25 c.c. or 50 c.c. pyrex centrifuge tube. The reaction mixture is allowed to stand *in the dark* for five minutes and then

spun at high speed for five minutes. Before decanting the clear supernatant fluid, it is best to ascertain that precipitation has been complete by adding a drop of the silver lactate solution to the supernatant fluid. After decanting the clear supernatant fluid, 6 c.c. of LiCl solution are added, and the mixture is thoroughly stirred with a fine glass rod. When the precipitate is equally divided and all particles broken up, about 12 to 15 c.c. of water are added, the whole stirred well, and the rod washed down with about 2 c.c. of water. The tubes are spun immediately at high speed for ten minutes. The clear supernatant fluid is decanted into a 50 c.c. volumetric flask, water added to about 30 c.c.; next, 4 c.c. of the 15 per cent NaCN, followed by the reagent, added slowly with constant shaking. Allow to stand for at least twenty minutes and read against a 2 c.c. standard set up in the usual manner. Such a standard will suffice for a uric acid concentration ranging from 35 to 70 mg. per cent. For weaker concentrations a 1 c.c. standard must be used, for stronger concentrations a 3 c.c. standard. Using a 2 c.c. standard the calculation reduces to $\frac{\text{St.} \times 50}{\text{Rdg. X}}$ = mg. per cent. Since the color is stable for twenty-four hours and fully developed within twenty minutes, no difficulty is experienced if a stronger standard is found necessary. It is best to dilute 1:10 urines which contain a large amount of uric acid and to use 2 c.c. of this dilute urine for the determination, thus employing 0.2 c.c. instead of 0.4 c.c. of urine.

The results of the analyses given in Tables III and IV prompted us to carry out experiments to determine to what extent the presence of phenol, polyhydroxi-phenols, or certain amino-acids will increase the blue color of the reaction. For this purpose known amounts of phenol, of resorcinol, or of tyrosine, were added to aliquot parts of blood or plasma. The uric acid determinations were also carried out on control bloods or plasmas by the direct as well as by the isolation method. The results of these examinations are given in Table IV.

The procedure employed was briefly as follows: The blood or plasma was divided into aliquot parts of 5 or 8 c.c. On one such part the analyses were carried out in the usual manner, thus serving as control. To one of the other parts phenol in water solution was added, to another tyrosine in N/HCl solution, and to the last resorcinol in water solution. The volume of the addition, never exceeding 1 c.c. in the case of tyrosine or 3 c.c. in the case of phenol or resorcinol, was deducted from the water dilution used in the Folin protein precipitation method. Control experiments proved that the addition of N/10 HCl in the amounts used, bringing the final solution to at most 0.012 N with respect to HCl, does not interfere with the determination of uric acid. Whenever possible, 10 c.c. of the filtrate were used for the direct method and 15 or 20 c.c. for the isolation method.

While resorcinol as well as tyrosine gives a distinct blue color with the reagents, the color is not of sufficient depth to account for the difference found between the direct and the indirect or isolation method (Tables III and IV) unless we assume a great increase in the concentration of these substances ordinarily present in very small concentrations only. The blue color

TABLE IV

EFFECT OF THE ADDITION OF PHENOL, RESORCINOL OR TYROSINE ON THE COLORIMETRIC DETERMINATION OF URIC ACID IN WHOLE BLOOD

Addition to Whole Blood		Folin's Reagent: Determined Uric Acid mg. %		Folin's Reagent: Increase in Uric Acid Determined by Direct Method, Expressed in mg. % Uric Acid per mg. % of Addition			Benedict's Reagent: Determined Uric Acid mg. %		Benedict's Reagent: Increase in Uric Acid Determined by Direct Method, Expressed in mg. % Uric Acid per mg. % of Addition		
Substance	mg. %	Direct	Isolated	Phenol	Resorcinol	Tyrosine	Direct	Isolated	Phenol	Resorcinol	Tyrosine
No Addition		4.25	2.45	—	—	—	5.24	2.50	—	—	—
Phenol	3.6	5.00	2.50	0.21	—	—	6.75	2.50	0.31	—	—
Resorcinol	2.9	5.32	2.60	—	0.17	—	5.70	2.65	—	0.16	—
Tyrosine	1.0	4.25	2.50	—	—	0.00	5.55	2.50	—	—	0.31
No Addition		3.34	2.00	—	—	—	4.00	2.20	—	—	—
Phenol	2.25	3.75	2.10	0.18	—	—	4.70	—	0.31	—	—
Resorcinol	2.32	3.70	2.00	—	0.15	—	4.55	2.20	—	0.23	—
Tyrosine	1.00	3.34	2.00	—	—	0.00	4.35	2.10	—	—	0.35
No Addition		3.30	1.80	—	—	—	3.75	2.00	—	—	—
Phenol	3.00	4.00	—	0.23	—	—	4.65	—	0.30	—	—
Resorcinol	2.32	3.85	—	—	0.24	—	4.20	—	—	0.19	—
Tyrosine	1.0	3.35	—	—	—	0.05	4.05	—	—	—	0.30
No Additions		4.68	2.73	—	—	—	6.45	2.80	—	—	—
Phenol	3.6	5.40	2.80	0.22	—	—	6.75	2.80	0.36	—	—
Resorcinol	2.90	5.10	2.85	—	0.17	—	—	—	—	—	—
No Additions		2.92	—	—	—	—	4.55	—	—	—	—
Resorcinol	2.90	3.50	—	—	0.20	—	5.10	—	—	0.19	
Phenol	3.6	3.80	—	0.24	—	—	5.75	—	0.33	—	—
Averages				0.21	0.19				0.32	0.19	0.32

given by any of the three substances employed varies in tint from the clear blue color due to the presence of uric acid, and develops much more gradually. It is fully developed within an hour. The color given by phenol is the deepest. One mg. of phenol will give a color equivalent to 0.24 mg. uric acid with the Folin reagent and with the Benedict reagent will give a color equivalent to 0.37 mg. uric acid. The concentration of phenols in the blood varies between 2.5 to 5 mg. per cent. If present in the same concentration as uric acid, it will increase the uric acid value determined by the direct method by 24 to 37 per cent, depending upon the reagent used. If the readings are made within twenty minutes after the reagent has been added, the increase will not be quite as marked. Because of the grayish tinge imparted to the blue color by any of these substances, a difference in shade exists between the standard solution and the blood filtrate solution which renders the colorimetric reading more difficult. The color values of these substances expressed in mg. of uric acid per mg. of substance are shown in Table V.

TABLE V

	FOLIN REAGENT	BENEDICT REAGENT
1 mg. tyrosine	0.10 mg.	0.35 mg.
1 " resorcinol	0.16 "	0.25 "
1 " phenol	0.24 "	0.37 "

These values are the average values of six determinations made on aqueous solutions of these substances and are not based on the increases obtained by the direct method when these substances were added to blood or plasma.

CONCLUSIONS

Inspection of Tables III, IV and V leads to the following conclusions:

1. Substances are present in the protein-free blood or plasma filtrate which are not precipitated out by silver lactate or by basic zinc carbonate. These substances give a blue color with the Folin, as well as the Benedict uric acid reagent.

2. The concentration of these substances varies greatly.

3. They react with different intensity with the two reagents.

4. Phenol, resorcinol, or tyrosine if present will intensify the blue color produced by uric acid with the Benedict reagent. Phenol and resorcinol also react, though to a less extent, with the Folin reagent, but tyrosine gives only a slight color with the Folin reagent.

5. The preliminary isolation of uric acid as a silver salt does not cause an appreciable error in the determination of the uric acid in the blood. The loss that does occur, occurs in the precipitation of the blood or plasma proteins through adsorption on the precipitate. Since the precipitate is far less in case plasma is employed, the loss is also less. The loss occurs during precipitation; this fact is shown by the determination of uric acid of known concentration in a solution of sulpho-tungstic acid of the approximate concentration in the filtrates. This is further substantiated by the analyses carried out on blood and plasma with added uric acid. The percentage of recovered uric acid, determined by the isolation method, agrees within the limits of the experi-

mental error of the method with the percentage recovered as determined by the direct method.

6. No parallelism can be established between pathologic condition and concentration of the unknown substances which also react with the uric acid reagent. The group of pernicious vomiting cases is the only one showing a consistency in the divergence between the two methods.

SUMMARY

The direct method of uric acid determination on blood or plasma filtrate, using either Benedict's or Folin's reagent, invariably gives too high a result. It is shown that either of these two reagents gives a blue color with resorcinol and with phenol. The Benedict reagent gives a color with tyrosine as well. Neither of these three substances is precipitated by silver lactate, and there is no increase in the result of the uric acid determination carried out by the preliminary isolation method on bloods or plasmas to which either of the three substances has been added. In the great majority of cases neither phenol, resorcinol, or tyrosine is present in the blood in sufficiently high concentration to account for the total divergence between the two methods. No definite parallelism between pathologic condition and degree of difference has been observed. A modification of the Folin method of uric acid determination, which eliminates the danger of precipitation in the reaction mixture and produces a color which is stable for twenty-four hours, is described; and the method is adapted to the determination of uric acid present in the urine.

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THE SEDIMENTATION REACTION OF ERYTHROCYTES*

Clinical Applications and a Micromethod

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IN THE past few years much interest has been displayed by foreign clinicians and investigators in the study of the phenomenon known as the sedimentation reaction or the suspension stability reaction of the erythrocytes. When fresh blood is treated with an anticoagulative substance and allowed to stand in a column, the erythrocytes settle down, leaving clear plasma above. The extent and velocity of this sedimentation have been found to vary widely under certain physiologic and pathologic conditions.

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While credit for the discovery of this phenomenon and for its employment as a clinical test is generally given to Fahraeus,[1] who published a monograph on the subject in 1918, it has been pointed out by several authors that the phenomenon was described by Galen as the crusta phlogistica and is mentioned in English and German textbooks of the first half of the Nineteenth Century. Fahraeus was apparently unaware of this fact and also of the excellent papers beginning with that of 1894, published by Prof. Biernacki[2] of Warsaw on the same subject.

Biernacki prevented coagulation by the addition of powdered oxalate and made his determinations by placing the blood in specially constructed cylinders and measuring the degree of fall of the erythrocytes at thirty minutes and one hour. In 1897 he reported a series of sedimentation tests on seventy-five cases, including four of pulmonary tuberculosis, and tabulated his comparative results with defibrinated and oxalated blood. His conclusions were: that the test had clinical value, that the results were not dependent on corpuscular volume, and that the sedimentation velocity was related to the content of fibrinogen in the plasma and intimately related to conditions of disordered oxidation. Biernacki's work was confirmed by that of his pupil Müller[3] and reviewed by Marcano[4] in his study of corpuscular volumes.

Although the work of Biernacki clearly antedates that of Fahraeus, the latter author originated many important studies both on causative factors and on clinical application, and his papers have stimulated interest in the subject while the publications of Biernacki have passed apparently unnoticed, although reference is found to his work in one of Linzenmeier's[5] excellent papers.

CLINICAL APPLICATIONS

Clinically, the reaction has been employed in the study of a diverse group of pathologic conditions. As it was first suggested as a test for pregnancy, it has naturally been used rather extensively in obstetric clinics. It has been found that the reaction remains within normal limits until gestation has reached about the fourth month when it shows a consistent and marked acceleration of sedimentation velocity. As other and more familiar signs of pregnancy are not lacking at this period, its usefulness as a diagnostic method is slight. It has, however, been employed by some authors as an aid in diagnosis between large pelvic tumors such as myomata and ovarian cysts and pregnancy, as benign pelvic tumors do not alter the normal sedimentation reaction.

The test has had very extensive employment in gynecologic practice and has been found useful in distinguishing between pelvic inflammatory conditions and benign growths (Schumacher and Vogel,[6] Pewny[7]). Rumpf[8] considers that the test offers a valuable means to determine when surgical measures should be employed in inflammatory disease of the pelvis, as during the acute stages the velocity is markedly increased and tends to lower values with chronicity.

In surgery it has been suggested and employed with somewhat doubtful success in differentiating between acute appendicitis and acute adnexal dis-

ease. In the former the test is found to remain practically normal for about the first thirty hours and to be always markedly increased in the latter. (Joseph and Marcus,[9] Haller.[10]) W. Löhr,[11] who has used the test extensively, feels that it is of decided value in surgical diagnosis, that it offers a reliable indication of the severity and acuteness of any inflammatory lesion, and that it may be useful in distinguishing benign from malignant tumors. In this last observation he is confirmed by several authors (Gragert,[12] Haller[10]).

In pediatrics the test has found employment. Gyorgy[13] found that infants of over one month showed a somewhat more rapid velocity of sedimentation than adults. In infants of under one month, on the other hand, there was a definite retardation. This retardation has also been noted by Nadolny.[14] Gyorgy found that any fever process in infants was accompanied by an increase in the sedimentation reaction, but that if other causes could be excluded, a marked increase in velocity could be considered diagnostic of syphilis, as its occurrence was paralleled by a positive Wassermann and Sachs-Georgi reaction and diminished with successful antiluetic treatment.

In the field of general medicine the test has not proved very valuable, as most pathologic conditions associated with fever give an increased sedimentation reaction. As a diagnostic help in the differential diagnosis between ulcer and cancer of the stomach, it has been recommended by Löhr,[11] Hoffgard,[15] Kovács.[16] It seems always increased in florid syphilis and is definitely above normal limits in tabes and general paresis (Paulian,[17] Plaut[18]).

It is in the field of tuberculosis, however, that the test has had widest application, and reports of the study of about 17,000 cases of tuberculosis by this method are on record. In general the opinion of the value of the test is that it offers considerable information as to the activity and extent of the lesion present in the lung. The test has been used routinely for the last year and a half at Gaylord Farm Sanatorium, and a preliminary report of the results obtained has been made by one of the present authors (Morriss[19]). Further experience has added to our interest, and our present records of four hundred cases, with examinations repeated at varying time intervals on one hundred thirty cases, have shown such close agreement with other clinical indications that we have come to attach considerable importance to the test in our estimate of the condition of the patient and his need of treatment.

TECHNIC

An estimate of the value of the test has been confused by a lack of standardization in the methods employed and by the difference in terms used in expressing the results. Fahraeus[1] employs test tubes 17 cm. in length with an internal diameter of 9 mm. as containers of the citrated blood and states the results in millimeters (the measure of the fall of the level of erythrocytes at stated times). Linzenmeier[20] uses short tubes of 6.5 cm. length and 5 mm. internal diameter as containers, giving his results in minutes, an expression of the time within which the top of the column of erythrocytes sinks from the filling mark to a second mark placed empirically 18 mm. lower on the tube. Westergren[21] states his results in mm. but uses thin tubes sealed

at one end, of 30 cm. in length and 2.5 mm. internal diameter, and takes his readings at one, two, and twenty-four hours. Several other authors have proposed certain modifications in technic, but the three methods described are the ones usually employed. In all of the methods the blood is obtained from the arm vein and citrated before the readings are made.

The method employed in routine tests made at Gaylord Farm Sanatorium has been a modified Westergren technic, as described in detail in a preceding article (Morriss[19]). The readings are obtained at one, two, and twenty-four hours from ordinary 1 c.c. serologic pipettes graduated from 1 to 100, filled with citrated blood, and represent the percentage of clear plasma lying above the level of erythrocytes. As the pipettes are filled to exactly the 1 c.c. mark, the reading can be made directly from the pipette graduations, thus avoiding the necessity of measuring the results on a mm. scale. The advantages of a reading thus expressed in percentage also has been emphasized by Fischel.[22] In this technic about 2 c.c. of blood is necessary; it is obtained by venous puncture and diluted with one-fifth volume of 3.8 per cent of sodium citrate.

MICROMETHOD

While venous puncture is usually easy, it was obvious that the value of the test was limited considerably by the necessity of its employment both on account of the lack of familiarity on the part of many physicians with this method of obtaining blood, and on account of the patient's objection to its frequent application. There are also a few cases in which successful venous puncture is very difficult owing to small veins embedded in much subcutaneous fat, or on account of the extreme delicacy of the veins, such as are found in children and infants. Early in our work with the test we turned our attention to the feasibility of devising a micromethod that could be used with small portions of blood, such as were obtainable from the capillaries of the finger tip or ear.

The same need for a micromethod has been felt by several investigators, thus Biernacki[23] has described such a method in which he employed thermometer tubing of ½ mm. internal diameter and used powdered oxalate as an anticoagulant. Marcano[4] in 1901 published perhaps the first micromethod in describing an apparatus which he devised for determining relative corpuscular and plasma volumes, mentioning that it could also be used for determining Biernacki's sedimentation reaction. Fahraeus is quoted by Levinson[24] as having used a capillary tube which required only a drop of blood and yielded results roughly comparable with macromethods. Somewhat similar methods are described by Von Brinckman and Wastl.[25] Balachowsky[26] employed hirudin in a micromethod as an anticoagulant and was unfortunately deterred from verifying his results by the difficulty in procuring this substance. Much more recently methods have been described by Linzenmeier[27] and Kaufman.[28] The former employed a long slender pipette 27 cm. in length and of internal diameter of 1 mm. with a small mixing bulb placed in the center. He diluted with one-fifth volume of sodium citrate mixed by inverting the tube three times and made readings direct from the tube, the mixture being

allowed to settle into the lower half. The test required only two drops of blood and is described as reliable by its author. Kaufman's method is somewhat similar in principle.

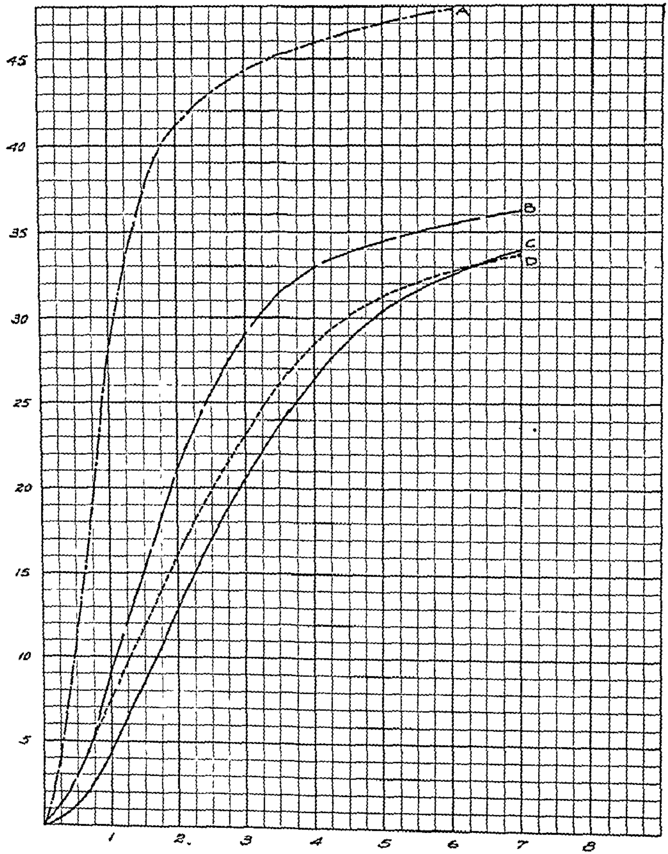

Fig 1.—A comparison of sedimentation curves in tubes of varying diameter. *A*, Capillary tubing. *B*, 0 1 c.c. pipette. *C*, 1 c.c pipette. *D*, 0 2 c.c. pipette.
Ordinates represent percentage sedimentation.
Abscissae fifteen-minute time intervals.

In devising such a micromethod two points required definite answer: whether tubes with a very narrow lumen could be employed, and whether blood obtained from capillaries would give results comparable to venous blood. A series of tests was made, using tubes of various diameters such as

0.2 c.c., 0.1 c.c., pipettes and capillary tubing. It was found that the smaller tubes gave a slightly higher percentage reading than the standard method, the differences increasing with the decrease in diameter of the tube; capillary tubes were unsatisfactory, but tubes of a caliber of 0.1 c.c. pipettes gave comparable readings.

Fig. 1 represents a comparison of sedimentation curves derived from tubes of varying diameters: *A*, capillary tubing; *B*, 0.1 c.c. pipette; *C*, 1 c.c. pipette; *D*, 0.2 c.c. pipette. The tubes were filled with the same citrated venous blood. A number of similar results convinced us that the test could be satisfactorily carried out in small tubes employing only 0.1 c.c. of blood. A series of tests using venous blood and finger blood set up in 0.1 c.c. pipettes yielded results in close enough agreement to establish the second point (Table I).

It remained to construct a pipette in which accurate dilution and satisfactory mixture of blood and diluting fluid could be obtained. The pipette devised is illustrated in Fig. 2.* The technic employed in its use is as follows:

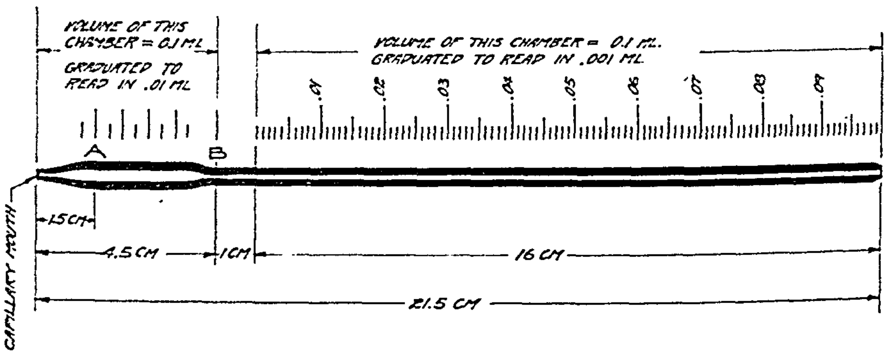

Fig. 2.—Pipette for microsedimentation test.

The patient's fingers are bathed for a few minutes in hot water to produce vasodilatation; the pipette is filled from the bulb end to mark *A*, or to one-fifth of the capacity of the upper calibration with 3.8 per cent sodium citrate; a large drop of blood is obtained from the finger and drawn up to the mark *B* on the diagram. It is important that free bleeding take place so that the blood can be obtained with little or no pressure. The pipette is then inverted and the blood allowed to run down into the lower end. To promote thorough mixing, which is essential, the mixture is then driven back into the bulb by gentle pressure through the mouthpiece and rubber tubing; the process is repeated three times. The pipette is then placed in a rack with spring clamps holding it in position. Readings are made direct from the scale as in the standard technic.

COMPARISON OF MICROMETHOD AND MACROMETHOD

In order to determine the reliability of the micromethod a series of comparative readings were made. Table II represents a comparison of averages in one hundred cases between the micro and the standard methods. The determinations were made by both authors to avoid the personal element in

*Pipette was made up through the courtesy of Arthur H. Thomas Company.

technic. A certain amount of practice in the use of the small pipette was found necessary before satisfactory results could be obtained, but it is a far simpler proceeding than the usual blood count.

It was found that slightly higher percentages were obtained with the micromethod, but that the differences were fairly constant and appeared to depend more on certain physical factors due to the tube caliber than on differences between capillary and venous blood.

TABLE I

COMPARISON VENOUS AND CAPILLARY BLOOD
Figures Represent Percentage Sedimentation as Read from 0.1 c.c. Pipettes

SERIAL NUMBER	1 HOUR		2 HOUR		24 HOUR	
	VENOUS	CAPILLARY	VENOUS	CAPILLARY	VENOUS	CAPILLARY
1	15	11	29	26	50	58
2	18	12	31	27	54	61
3	7	5	18	17	38	55
4	14	8	24	21	47	60
5	17	—	32	37	52	62
6	2	1	5	6	37	51
7	1	—	3	4	28	41
8	30	—	45	55	58	68
9	1	2	2	3	35	43
10	23	—	39	57	57	66
11	7	15	21	29	47	54
12	44	47	58	59	68	68
13	—	14	24	33	55	58
14	—	2	7	3	41	50
15	33	42	50	59	—	—
Average Difference	4.4		5		9.2	

TABLE II

A COMPARISON BETWEEN THE MICROMETHOD AND THE MACROMETHOD IN 103 CASES

READING TIME	NUMBER OF CASES	AVERAGE DIFFERENCE	MAXIMUM DIFFERENCE
1 hour	69	4.9	15.5
2 "	103	7.6	23.5
24 "	71	10.2	25.0

TABLE III

THE RELATIONSHIP BETWEEN SPEED OF SEDIMENTATION AND THE AVERAGE DIFFERENCES IN MICROMETHOD AND THE MACROMETHOD

SEDIMENTATION VALUES %	NUMBER OF CASES	AVERAGE DIFFERENCE
0–10	25	4.5
10–20	32	7.2
20–30	21	9.3
30+	25	9.9

In rapidly sedimenting blood the percentage of difference was increased as shown in Table III. While on the whole the micromethod is not as reliable as the standard macromethod, it is sufficiently so to be employed with confidence for clinical purposes and can be translated into results comparable with the regular technic. It thus offers a method of wider general application than that employing venous puncture and enables us to study the reaction when frequently repeated tests seem necessary.

CONCLUSIONS

1. The sedimentation reaction has found widespread employment as a clinical test and has been found especially useful in tuberculosis as an indication of the degree of activity present.

2. A micromethod is presented employing only 0.1 c.c. of blood which offers results comparable to standard macromethods.

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MICROSCOPIC STUDIES ON CAPILLARY INNERVATION AND STAINING OF THE ENDOTHELIAL CELLS*

By Irving S. Barksdale, M.D., Charleston, S. C.

RECENT investigations on the blood capillaries by Krogh, Dale and Laidlaw, Danzer and Hooker, Richards, Boas, Carrier, and others are calling much attention to this field and are serving as a great source of inspiration for further work along clinical lines. No doubt some of the researches have been quite overenthusiastic, especially the work done by the Germans in their studies on the human capillaries as pointed out by Boas. However, something of value may be gleaned from further observations.

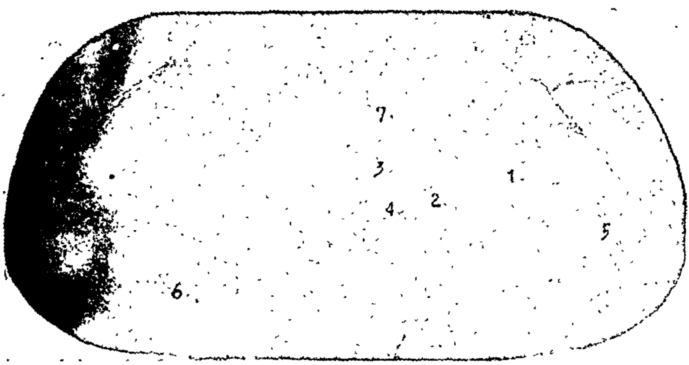

Fig. 1.—Photomicrograph of the frog's tongue (x 160). *1* Nerve fibril. *2*. Contact of blood capillary with nerve fibril. *3*, *4*, and *7*. Network of capillaries in contact with branches from the nerve fibril (*1*). *5*. Arteriole. *6*. Venule. *8*. Vein.

INNERVATION OF THE CAPILLARIES AND ARTERIOLES IN THE FROG

Krogh has demonstrated that cocainization abolishes the responses of capillaries to mechanical, as well as to certain chemical stimuli and has suggested a nervous mechanism due in all probability to axone reflexes. Bruce showed that oil of mustard applied to the skin causes a local cutaneous inflammation which persists after sectioning the dorsal roots of the spinal nerves; this fact suggests that the reaction must have something of an axone reflex nature and consequently could not be attributed to a spinal reflex. After the sensory fibers had degenerated, this effect could not be elicited.

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"A similar axone reflex has been found to occur in certain connector fibers of the thoracico-lumbar outflow which supply the smooth muscle of the bladder, the blood vessels of the rectum, and the internal anal sphincter. These fibers send collaterals to the bladder through the hypogastric nerves. When one of these is cut and stimulated centrally, the muscles innervated by the collaterals in the other hypogastric nerves are seen to respond. There is no evidence, however, that this mechanism is brought into play in normal life." Macleod.

Microscopically, it has been possible to study the distribution of nerve fibers in the frog's tongue and mesentery. With a magnification of 160 diameters larger nerve trunks were located and traced as far as possible to the point of branching. The branches were seen to approach one or more capillaries and touch, and beyond the points of contact they could be traced no

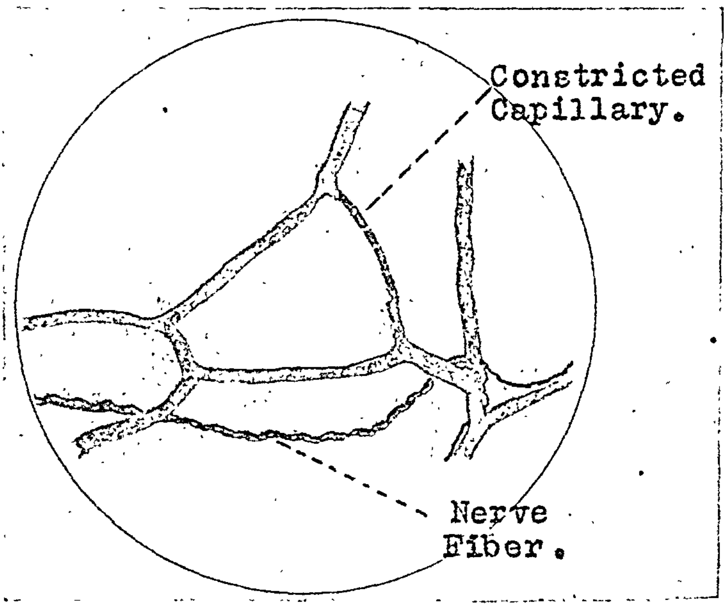

Fig. 2.—Innervation of the capillaries. From the frog's tongue (16 mm. objective, 10x eyepiece).

further. (See Figs. 1, 2, and 3.) Many arterioles, on the other hand, were seen to receive directly into the substance of their walls small collateral-like branches from the larger nerve trunks. (See Fig. 4.)

From these findings we can agree with Krogh that the capillaries are innervated, not necessarily by continuity but by contiguity. The nerve impulse enters the vessel at the point of contact with the nerve fibril and is propagated from there to other parts of the capillary and to other capillaries, the conducting medium being the walls of the vessels. The liability of confusing lymphatics with nerve fibers in the study of the frog's mesentery was borne in mind, and care was taken to differentiate between the two structures. Practically every individual axone process in the nerve fibers was discernible: comparatively few collateral branches are given off, there is a slight tendency towards translucency, and these are seen to pursue a more or

less tortuous course. The lymphatics, on the other hand, differ quite markedly from this description. There is less branching; they are transparent with sharply defined walls, are quite readily stretched, and in the spread mesentery follow a straight course.

STAINING OF THE CAPILLARY ENDOTHELIAL CELLS

Krogh has contributed much in the study of the capillary endothelial cells by outlining the internal surfaces following the injection of india ink. Guinea pigs received injections of the dye intravenously, were then killed and both the fresh and stained tissues examined microscopically, The capillary tubes were simply elongated dark fields, and both endothelial cells and blood corpuscles appeared in striking contrast to the surrounding black medium. Such a method might enable the investigator to make fairly accurate observations on the turgidity of the capillary wall during constriction and dilatation.

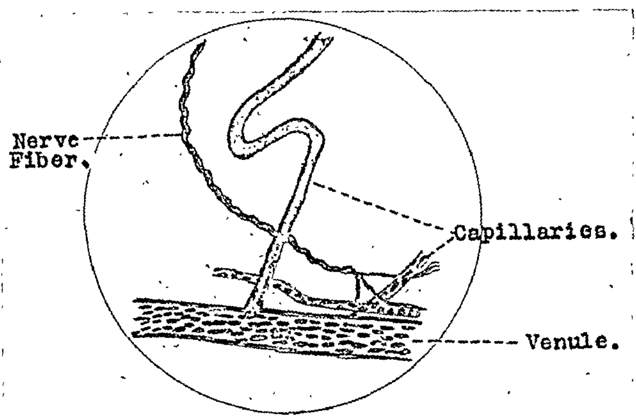

Fig. 3.—Innervation of the capillaries. From the mesentery of the frog (16 mm. objective, 6x eyepiece).

Leonard Hill placed a drop of 20 per cent urethane (ethyl carbamate) on a capillary field which dilated the capillaries and followed this up with an intravenous injection of neutral red. Shortly afterward the bordering tissues of the capillary were stained, with the stain fading away from the same capillary. Krogh thinks that this may be due to increased permeability of the wall. This method enables us to study the physiology of the endothelial cells lining the capillaries in relation to the possibility of their becoming thickened or thinned in capillary constriction and dilatation, in that the ill-defined external surfaces of the cells may be compared with the stained outlying tissues. Certain discrepancies, which are obvious, would arise in the attempt to study these during physiologic activity.

After numerous attempts at staining the endothelial cells in the capil-

laries of the frog, it has been found possible to stain them slightly intra vitam. A number of anilin dyes were tried, but these proved to be too toxic. However, a subcutaneous injection of 0.5 c.c. of a 1 per cent solution of indigocarmin will cause a marked bluing of the thighs and legs as well as a lesser amount of coloration of other parts of the body. At the same time the capillary walls are stained a pale bluish-gray. A number of capillary fields were studied in the web of the frog's foot: magnifications of 100 diameters revealed but little detail; magnifications of 440 diameters brought out the clearly outlined, pale bluish-gray endothelial cells. The most satisfactory method of study, however, was with a magnification of 950 diameters (oil immersion). The surrounding tissues were stained more deeply than the cells under observation. The cells were quite regular in outline except for some

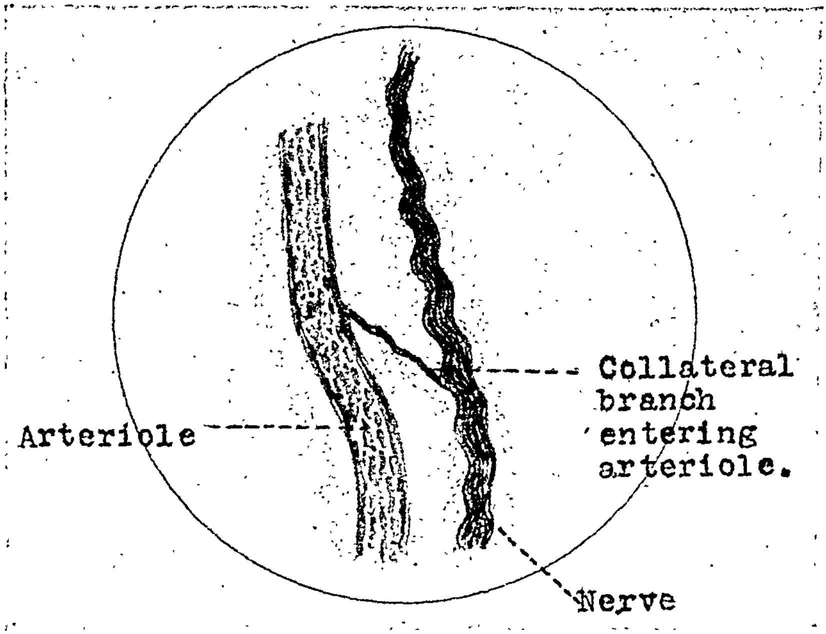

Fig. 4.—Innervation of arteriole. From the frog's tongue (16 mm. objective, 10x eyepiece).

bulging into the lumen of the capillary at short and irregular intervals. The external surface of the cell also presented occasional irregularities. Neither nuclei nor contiguous surfaces were discernible. Another satisfactory method of introducing the dye into the frog is by intracardial injection. No ill effects come from the slow introduction of 0.3 c.c. of a 1 per cent solution of indigocarmin directly through the ventricular wall with an ordinary hypodermic needle. The heart rate is accelerated and the capillaries are slightly dilated after the administration of the dye. With this method of study, no thickening of the endothelium lining the capillaries was observed when they were made to constrict. This disproves the cell-turgor theory which has been advanced to explain capillary constriction and dilatation. As will be recalled, the theory states that in active constriction of the blood capillary, the endothelial cells lining the lumen are capable of imbibing water either from the

blood stream or from the outlying tissues, at the same time becoming so thickened as to encroach inwardly, thereby lessening the diameter of the lumen. Dilatation was explained by the loss of water from the endothelial cell, which in turn caused a thinning out of the cell with the concomitant increase in the diameter of the capillary lumen.

SUMMARY AND CONCLUSIONS

The blood capillaries in the frog's tongue are innervated by contiguity with the nerve fibrils rather than by continuity with them.

The arterioles, on the other hand are innervated not by contiguity, but by continuity with the nerve fibrils.

Five-tenths cubic centimeter of a 1 per cent solution of indigocarmin injected subcutaneously stains the capillary endothelium a bluish-gray color. The same effect may be obtained with an intracardial injection of 0.3 c.c. of the same solution.

Examination of the stained capillary endothelium with the oil immersion lens reveals neither a thickening of the cells during capillary constriction nor a thinning out during dilatation.

MERCUROCHROME IN TREATMENT OF TYPHOID FEVER: A REPORT OF THREE CASES WITH COMPLICATIONS*

By John H. Foster, M.D., and H. A. Chao, M.D., Changsha, China

SOME remarkable results have been reported following the intravenous administration of mercurochrome-220 soluble in various types of infections and infectious diseases, apparently demonstrating that a valuable remedy has been added to therapeutics.[1]

From its demonstrated antiseptic action in the blood, in the bile, and in the intestinal tract we were led to hope that mercurochome would be of value in the treatment of typhoid fever. Furthermore the reaction after the intravenous injection of mercurochrome rather closely resembles the response to foreign protein, which is beneficial in a certain percentage of typhoid fever cases.[2] Typhoid and paratyphoid fevers are quite prevalent in Changsha during the summer and fall months, and the mortality has varied from 10 to 17 per cent in different years. Some more active and effective form of therapeutics than the present dietetic treatment would be warmly welcomed.

We selected two cases of typhoid fever of moderate severity and free from complications and gave to each 5 mg. of mercurochrome-220 soluble per kilogram of body weight in a freshly prepared 1 per cent solution, with the following results:

Case 1.—T. M. L., a student, aged twenty-four years, was admitted on October 24, 1924, on the sixth day of his illness, with symptoms and signs of typhoid. Blood culture

*From the Medical Service of the Hunan-Yale Hospital.
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and Widal were positive to typhosus. A parotitis developed which subsided in about two weeks. He was given two intramuscular injections of milk with good reactions but with no effect on the temperature curve. On November 6, the thirty-eighth day of his disease, he was given an intravenous injection of 20 c.c. of 1 per cent mercurochrome-220, at 2:30 P.M. Two hours later he had a chill and complained of vertigo. The chill ceased at 6:30 P.M., but he had malaise and abdominal distress during the night. About noon the next day he had a loose bowel movement, colored by the dye, but consisting chiefly of blood and mucus, similar to a severe dysentery stool. There was no further hemorrhage, but the abdominal pain continued until late that afternoon. The temperature seemed to be lowered by the treatment (Chart 1), but did not remain normal until more than a week later. He left the hospital well after another week.

CASE 2.—T. M. M., a laborer, twenty-four years old, was admitted on November 1, 1924, with fever and headache of five days' duration. Symptoms and signs were typical of

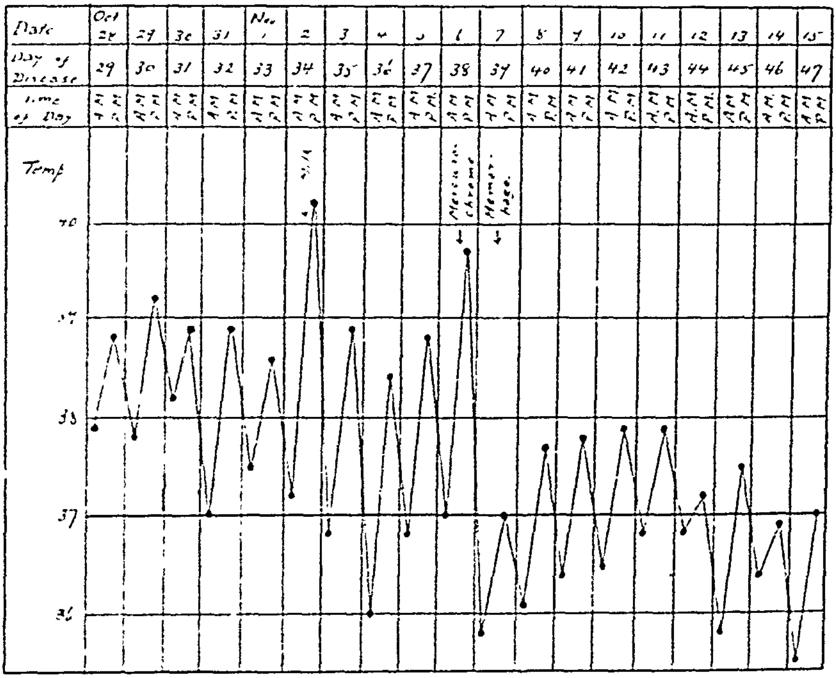

Chart 1.—Temperature chart of T.M.L. (Case 1). Intestinal hemorrhage twenty-two hours after administration of mercurochrome-220 soluble.

typhoid. The blood examination showed 8,200 leucocytes with 61 per cent polymorphonuclears and 39 per cent lymphocytes. Urine and feces were negative. Widal was positive, macroscopically, to typhoid on November 3. There was some abdominal pain at time of admission and he had some retention of urine for which he was catheterized on November 2 and 4. On November 6 at 3:40 P.M. he was given 21 c.c. of a 1 per cent mercurochrome-220 solution, equivalent to 5 mg. per kilogram of body weight. Thirty-five minutes later a chill began which lasted for over two hours. The temperature rose to 39.2° C. His leucocyte count during the chill dropped to 7,400. He complained of malaise and abdominal discomfort. At 9:00 A.M. the next day the temperature had dropped to 36.8° C., and the white cell count had risen to 17,400. At noon, about twenty hours after the injection, he complained of abdominal pain and had a rather profuse intestinal hemorrhage, about 400 c.c. or 500 c.c. of dark blood, with very little fecal matter or mucus. The pain became more intense and rigidity and tenderness appeared. The leucocyte count at 8:00 P.M. was 19,800 with 78 per cent polymorphonuclears. Perforation of the intestine was diagnosed and laparotomy performed about midnight. A perforation was found in the lower ileum. The patient's con-

dition remained critical for several days and he had two intestinal hemorrhages during the week after the operation. He eventually made a good recovery and left the hospital five weeks later (December 22).

With hesitancy we tried intravenous mercurochrome again, not entirely convinced that the two mishaps had been more than an accidental coincidence.

CASE 3.—C. F. C., a coolie, thirty-eight years of age, was admitted on November 9, 1924, with symptoms of eight days' duration. He had a palpable spleen. Rose spots were present, and he had signs of moderate bronchitis. His leucocyte count was 5,800, with 54 per cent polymorphonuclears, 43 per cent lymphocytes and 3 per cent mononuclears. The Widal was first negative but later positive to paratyphoid-A. Mercurochrome-220, 5 mg. per kilogram of body weight, in a 1 per cent solution, was given at 2:30 P.M. on November 20. This was followed by a chill, as in the previous case, in about thirty minutes, but this time there was

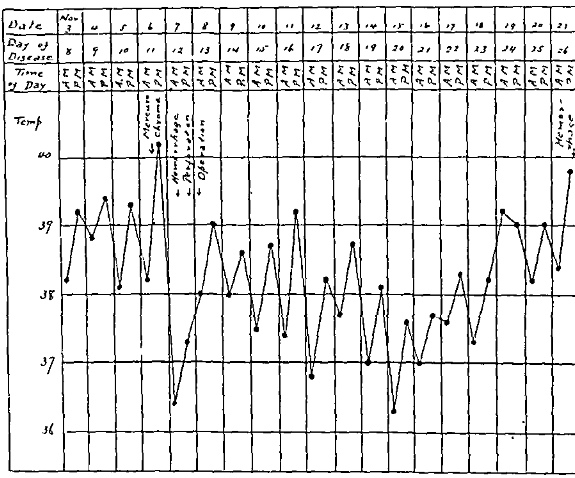

Chart 2.—Temperature chart of a typhoid fever patient (Case 2), in whom intestinal hemorrhage and intestinal perforation followed intravenous injection of mercurochrome -220 soluble.

nausea and vomiting. During the chill the white cell count rose to 13,800. That night the patient had two or three loose watery stools, which were colored by the dye, but which contained no blood. About eighteen hours after the injection he complained of abdominal distress. At noon he had a stool which was composed mostly of blood and mucus and at 8:00 P.M. he had a rather profuse hemorrhage. During the next four weeks a mild diarrhea, with one to four loose stools a day, persisted. Small amounts of blood were frequently present in the bowel movements. The patient left the hospital on December 15. We heard later that he died a few days afterwards following quite profuse intestinal hemorrhages.

The temperature charts of the three cases are presented to show the negligible effect of the injections on the temperature curves.

Leucocyte counts made before and after the injections of mercurochrome-220 are shown in Chart 4. It is interesting to note that in Case 2, the one in whom the perforation occurred, there was a leucopenia instead of the usual leucocytosis during the chill.

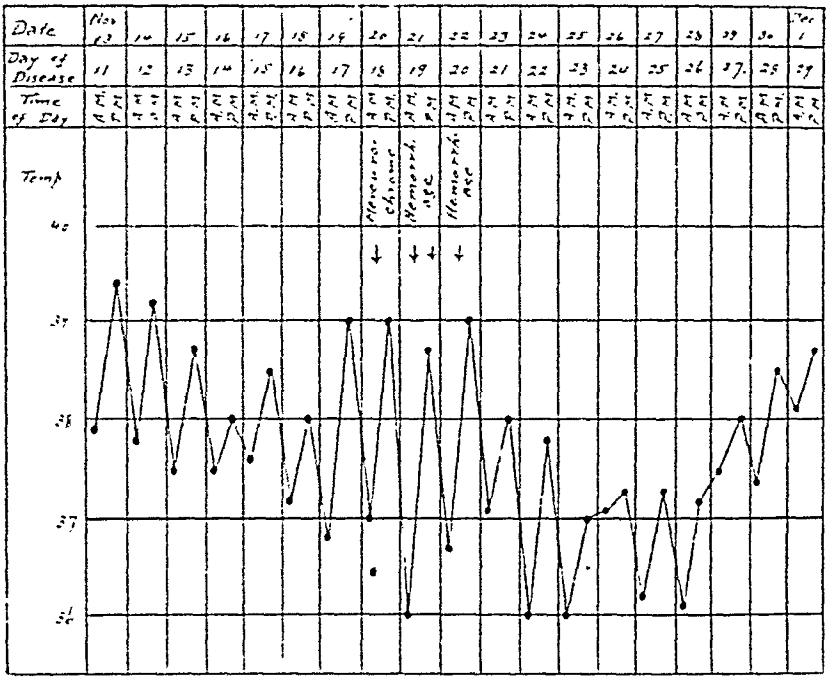

Chart 3.—Showing the temperature curve in the paratyphoid fever patient (Case 3, which terminated fatally), in whom hemorrhages followed mercurochrome treatment.

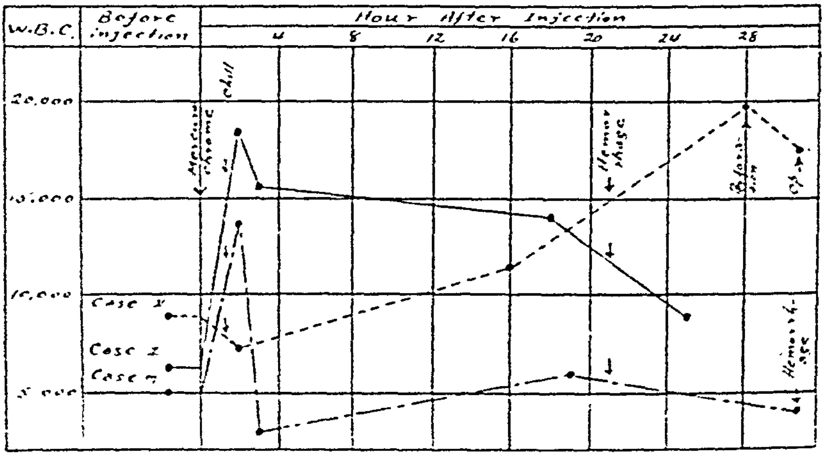

Chart 4.—Showing the leucocyte counts before and after the injection of mercurochrome-220 soluble in two cases of typhoid and one of paratyphoid fever.

SUMMARY

Intravenous injections of mercurochrome-220 soluble, in doses of 5 mg. per kilogram of body weight, were given to two cases of typhoid fever and one of paratyphoid, on the thirty-eighth, eleventh and the eighteenth day of the disease. In none of the cases was there any marked effect on the temperature curve. Two cases showed a temporary leucocytosis during the chill. In all three cases, following the injection, there was a chill with general malaise and abdominal distress. One had nausea and vomiting. About twenty hours after the treatment all three had an intestinal hemorrhage, moderate in one

but quite severe in the others. The first case had no further complications and was discharged in two weeks. The second case had a perforation of the intestine but recovered after operation. In the third there was continued diarrhea and the patient died about four weeks later with more hemorrhage.

COMMENT

These results are quite different from those so far reported for typhoid fever. In the twelve cases treated by Bond and Barrier[3] and in the seven additional cases reported by Young[1] no serious complications were noted.

Three cases are too few to be taken as the basis of any general conclusions, but the symptoms, occurring in three consecutive cases, at about the same interval after the injection and in such varying periods in the course of the disease, seem to be due to the drug. Possibly smaller doses would not have produced such symptoms. Young,[1] however, declares that severity of the diarrhea is due to individual variations in the patients and is not dependent upon the size of the dose. It seems to us that intravenous injections of mercurochrome-220 are contraindicated in typhoid fever and other cases with intestinal ulceration.

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THE STHENIC ACTION OF ADRENALIN ON THE INTESTINES*

By W. J. R. Heinekamp, Chicago, Illinois

THE most generally accepted action of adrenalin on the gastrointestinal tract is inhibition of peristalsis and relaxation of tone,[1-6] the sphincters being contracted.[7] Elliott,[8] using rabbits, and the balloon method for recording contractions, found that adrenalin relaxed the stomach to extreme flaccidity, while the pyloric sphincter contracted. Bech,[9] working with rings from the middle of the frog's stomach, found that strong solutions of adrenalin produced relaxation and decreased tonus. Exceptionally at the beginning, a contraction was effected. Hoskins and McClure,[10] using the balloon method, found that the small intestine was brought to a standstill and extreme dilatation was produced by quantities of adrenalin inadequate to cause a minimal sustained rise in blood pressure. O'Connor,[11] employing excised tissue, rabbits and doves, found that adrenalin effected inhibition of the intestines. Hirtz,[12] using strips of rabbit's pylorus, observed a relaxation with 1:200,000 adrenalin. Trendelenburg and Fleischhauer[13] found the gut to be inhibited even when no change in blood pressure was noted. Schuller,[14] using frog's rectum, noticed inhibition only with adrenalin. Tezner and Turold[15] state that adrenalin in concentration of 1:1,000,000 inhibited the pylorus, but, after acetylcholine, adrenalin effected contractions following an inhibition of short duration. Stross[16] found adrenalin to inhibit the rhythmic movements of frog's stomach (rings) as well as the *pendlebewegungen* and rhythmic fluctuations of dove intestine. Holm[17] never observed anything but inhibition of the human appendix following the use of adrenalin. Carlson[18] stated that adrenalin has a predominantly inhibitory action on the stomach of the cat and dog, and a motor action in the rabbit. He states also that the splanchnics, like the vagi, carry both motor and inhibitory efferents to the stomach.

A group of investigators have, however, found the opposite effect. Bunch[19] asserted that suprarenal extract freshly prepared, when injected into animals, always produced systolic tone of gut. Ott[20] also mentions this finding, but neither of these authors advance an explanation. Magnus[3] mentions a single instance in which increased peristalsis resulted from the use of adrenalin. Hoskins[21] states that epinephrin in remarkably high dilutions inhibits peristaltic activity of isolated segments of rabbit's intestine but that still higher dilutions augment often the rhythmic activity. He explains the augmentation produced as being due to stimulation of the metabolic processes of the muscle itself by quantities of the drug too small to affect the sympathetic myoneural junctions, the efficiency of the tissue being thereby increased. Smith[22] found that dilute solutions of adrenalin produced varying

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changes on various parts of the stomach of different animals. Gunn and Underhill[23] say that adrenalin relaxed the isolated small intestine of rabbits, but that it contracted the muscularis mucosae of cats' intestines. Tashiro[24] found segments of catgut suspended in Ringer's solutions to have increased tonus and sometimes augmented movement of the circular muscle following the use of adrenalin in dilute solutions. He thinks the drug acts on the motor sympathetics. Katsch,[25] using the x-ray, found adrenalin to effect motor inhibition of the gastrointestinal tract.

The augmentor effect of adrenalin is considered by Gruber[26] as due to that drug stimulating in weak solutions the augmentative nerve fibers arising from the thoracolumbar autonomic nervous system.

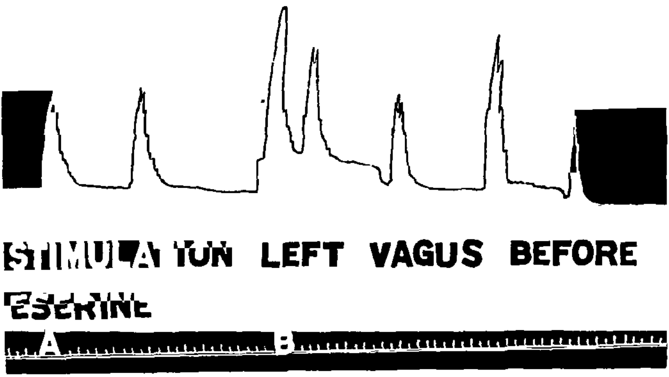

Fig. 1.—Change produced by stimulating intact cervical left vagus. Coil at 12 cm.

In general, the work to date, most of it on excised tissue of rabbit or frog, indicates: (1) that adrenalin ordinarily relaxes the gastrointestinal canal with the exception of the sphincters, which are contracted; and (2) that adrenalin in very minute quantities causes contraction of the gut. This is most generally explained by stating that adrenalin in very dilute solutions effects contractions by stimulating the motor sympathetics, and produces inhibition by stimulating the inhibitory sympathetics, the action in either case being on the myoneural junctions, as suggested by Dixon.[27]

In recent work[28] we found that adrenalin stimulated in a marked degree the peripheral parasympathetic apparatus to the heart. While working on intestinal peristalsis we have occasionally observed an augmentation in tone and an increase in peristalsis, following the intravenous injection of minute doses of adrenalin.

The present investigation was done to determine the cause of this sthenic action of adrenalin.

METHODS

In the experiments, well nourished dogs weighing from 10 to 15 kg., and under light ether anesthesia were used. The recording of respiration and blood pressure was by the usual methods. All drugs were injected intravenously through a cannula inserted into the femoral vein, and were washed in with 5 c.c. of warm physiologic saline. The stomach tracings were recorded by the modification of the Trendelenburg method described by Love.[29] It is

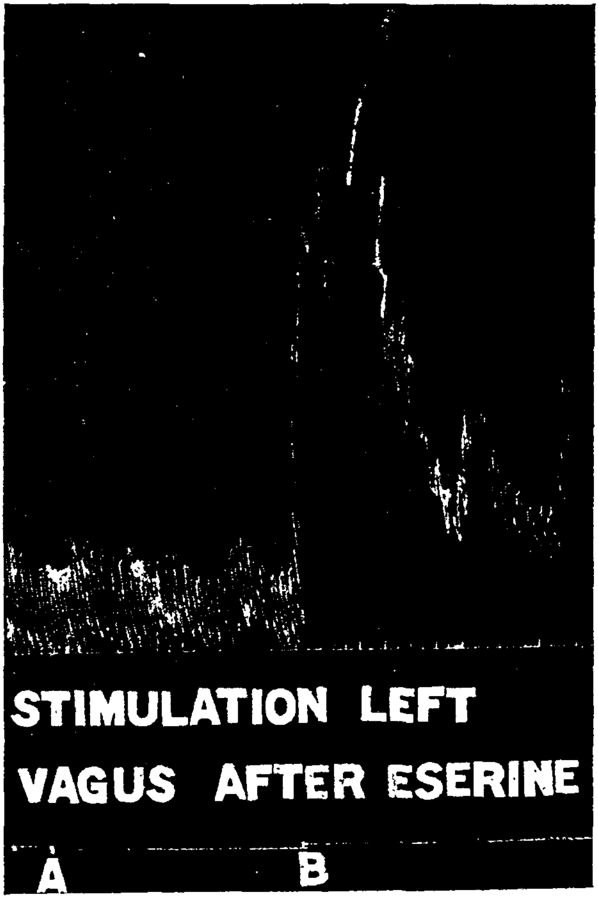

Fig. 2.—Change produced by stimulating intact left vagus after a sensitizing dose of eserine.

our opinion that this method is most simple, since the suturing may be carried on within the abdominal cavity, and the technic is very easily executed. The stomach does not chill and is not subjected to trauma. The animals were fed chopped meat about forty-five minutes before they were used. A portion on the anterior wall of the stomach about 2 inches from the pylorus was used. The stomach was always returned completely to the abdominal cavity and the opening in the belly wall closed as much as possible with sutures. Towels which were kept hot with water were wrapped around the pipe and covered the entire wound. In this manner the stomach did not become cold. The

vagi were usually cut in the cervical region, since we found the contractions to be more marked after this procedure. The splanchnics were left intact.

Electrical stimulation of the peripheral end of the cut vagus was usually carried out with the secondary coil at 12 cm. and the current furnished by a storage battery.

RESULTS

In general, our results were as follows: Large doses, e.g., one c.c. 1:10,000 of adrenalin, always effect a cessation of peristalsis (Fig. 3) and usually a de-

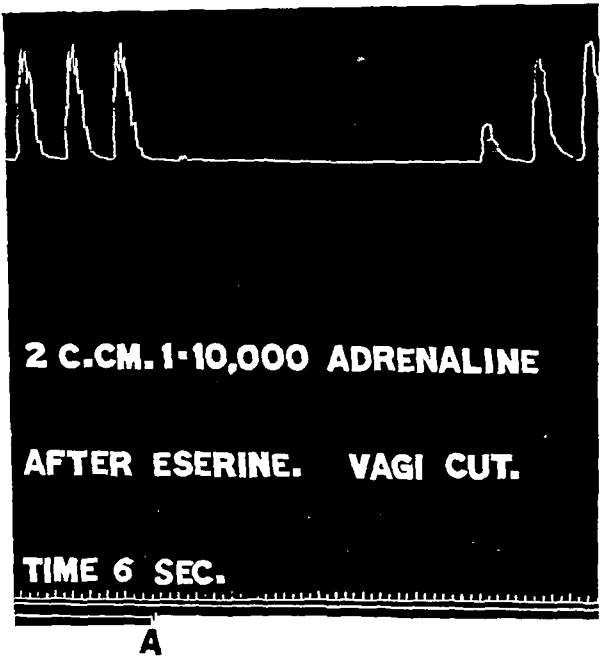

Fig 3.—Inhibition of peristalsis produced by adrenalin injected at point *A*.

crease in tone. Very minute doses, e.g., one c.c. 1:1,000,000, produce the same effect in many cases. This amount, however, usually produces an increase in tonus and often an increase in peristalsis that is of short duration (Fig. 4). *After small doses of eserine, e.g., ½ c.c. 0.1 per cent in a dog of 15 kg.*, small doses of adrenalin more frequently produce marked increase in tone and peristalsis (Fig. 5). It is usually of short duration, but in one experiment the action was prolonged (Fig. 6), and in another the gut became tonically con-

tracted for a period of three minutes, being relieved by atropine sulphate. In no experiment have I seen this action of adrenalin after the use of atropine.

Stimulation of the vagi after eserine effects a more marked increase in peristalsis and tone, indicating sensitization of the peripheral apparatus by the drug.

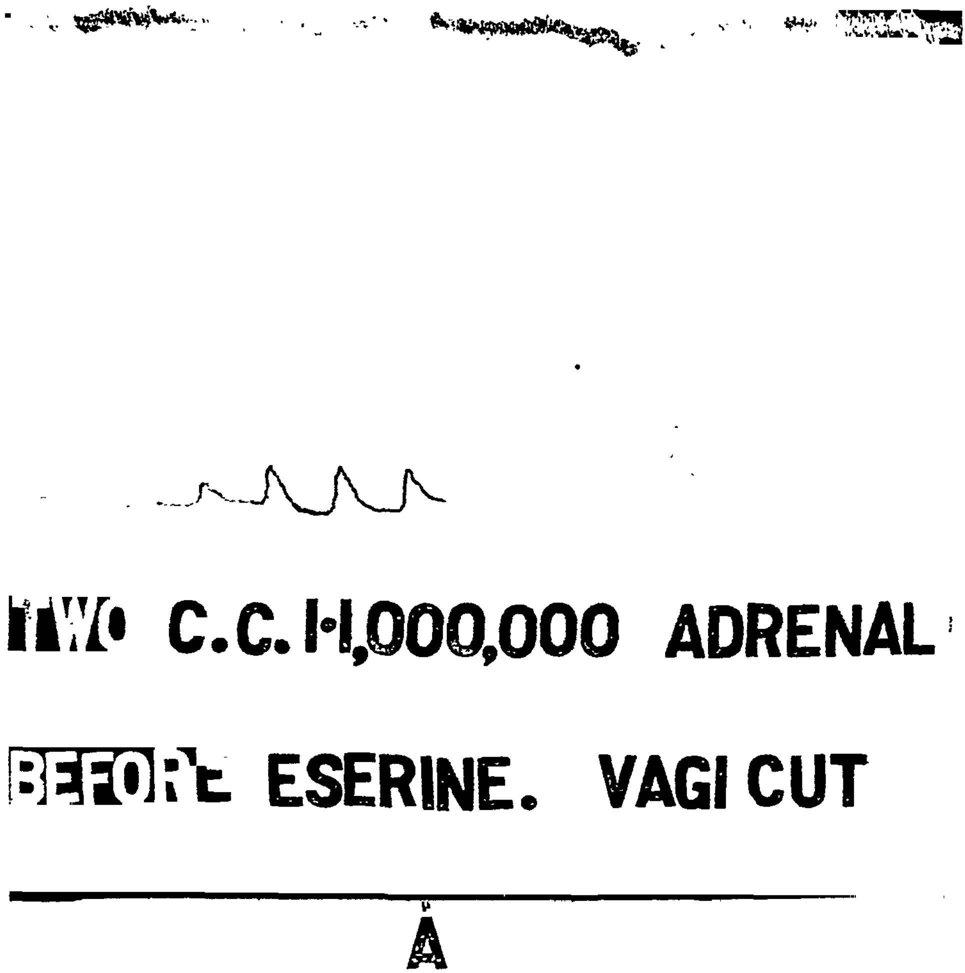

Fig. 4.—Change produced by adrenalin injected at point *A*. Note increased tonus and peristalsis.

DISCUSSION

Stimulation of the vagus nerve effects various results as far as the stomach is concerned. There may be a primary period of decreased tone and peristalsis followed by a secondary period of increased activity, the most common change. There may be no effect during stimulation followed by increased contractions after cessation of the stimuli, or there may be a marked increase in tone and peristalsis immediately on stimulating the vagus. We consider any one of these reactions normal, the latter being the less frequent.

In view of the fact that physostigmine causes contraction of plexus-free preparations, it is conceded that the drug acts upon the nerve endings.[30, 31] In 1915, Langley and Kato[32] found that physostigmine lowered the threshold of nerve excitation. In some previous work[28-33] we found the peripheral

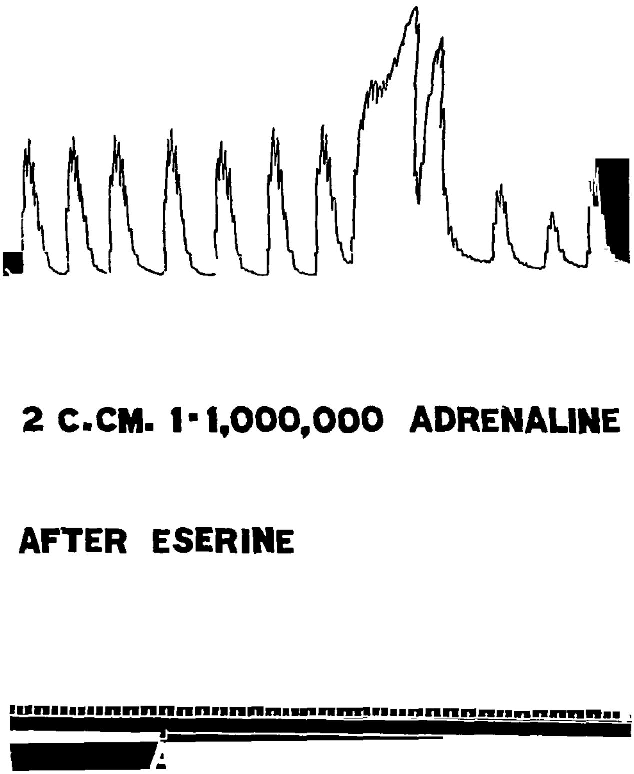

Fig. 5.—Change produced by adrenalin after section of both vagi, and after a sensitizing dose of eserine.

vagus apparatus to the heart sensitized, i.e., the threshold to electrical stimulation was lowered. We believe the vagus to the stomach to be similarly affected, in view of the fact that stimulation of the cervical vagus usually effects a greater reaction from the intestine after a small amount of eserine

has been administered than before. This effect may be seen following the use of 0.15 to 0.20 mg. per kg. of body weight, an amount which in itself produces no effect.

Adrenalin may effect inhibition by: (1) direct action upon the muscle; (2) stimulation of the motor sympathetics; or (3) stimulating the motor parasympathetics. In view of the fact that the sympathetics and parasympathetics are relayed through the metasympathetic system, i.e., the intrinsic nervous apparatus of the intestine, we shall consider the last as part of either of the other two systems.

The usual effect produced by adrenalin in a dose of 0.1 mg. is a marked inhibition of peristalsis and decrease in tone due to stimulation of the myoneural junctions of the sympathetic nerves, as proved by Dixon.[34] With small doses, however, as stated above, adrenalin actually produces an in-

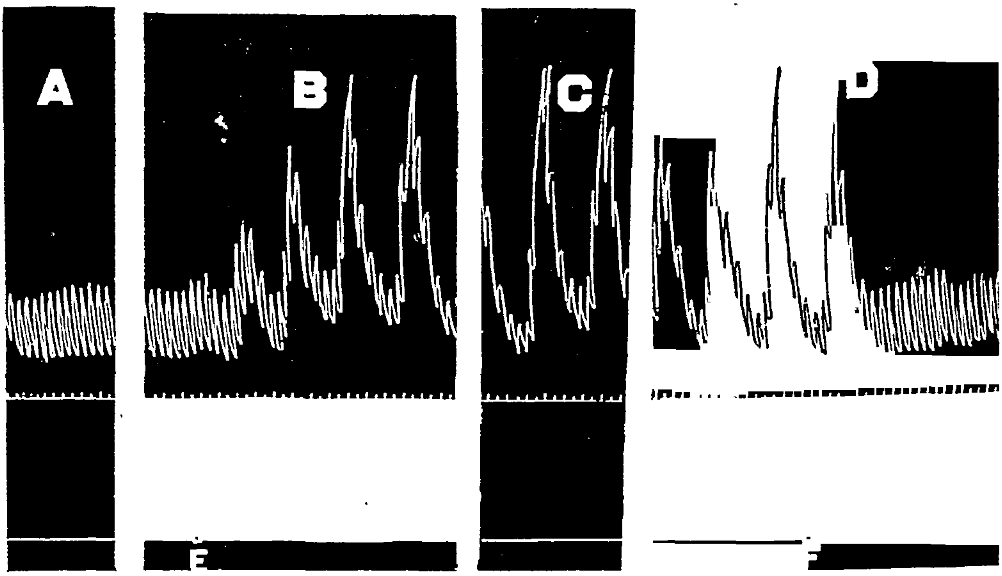

Fig. 6.—Changes effected by adrenalin and atropine. This was the most marked result obtained. *A*, before adrenalin; *B*, immediately after injection of adrenalin at *E*; *C*, three minutes after; *D*, change effected by injection of atropine at *F*.

creased activity of the intestines. This we think is not due to the stimulation of the motor sympathetics nor to direct changes in the muscle, since it is increased after eserine (synergy) and is totally prevented by atropine as shown in Fig. 5.

We do not believe that adrenalin produces inhibition by direct action of the muscle since it does not inhibit the contractions effected by barium chloride (Sollman, p. 331). Nor do we think it due to metabolic changes in the tissue, as advanced by Hoskins, for reasons amply given by Gruber.

In view of the fact that after small doses of eserine, contraction of the stomach is more active than before it, while stimulation of the thoracic sympathetic chain effects the same change, we believe that eserine in these amounts affects the parasympathetics only. And since this action of adrenalin is promptly relieved by amounts of atropine which render the vagus inactive

to electrical stimulation, we believe that the site of action is the peripheral parasympathetic apparatus.

It is our opinion that the effect, whether sympathetic or parasympathetic, depends upon the amount of adrenalin used. We believe that adrenalin stimulates both the parasympathetics and the sympathetics simultaneously, the latter more markedly. The threshold of the motor nerves, however, is lower to adrenalin activity, but its maximal reaction is much less than that of the inhibitor nerves. That is, a dose subminimal for the sympathetics will exert a maximal effect upon the vagus, but a maximal dose for the sympathetics exerts the same maximal parasympathetic change that a smaller dose will.

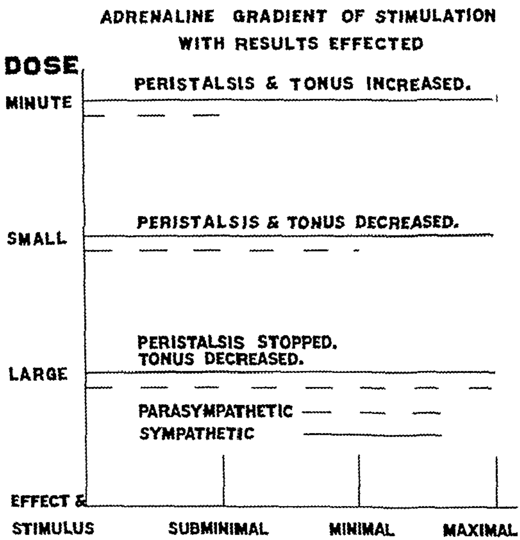

Chart I.

Increasing doses do not effect a proportionally greater reaction from the vagus, as they do from the sympathetics. While it takes a greater amount of adrenalin to act on the inhibitors than on the activators, a more marked reaction is obtained when the action of the latter is obtained. Thus, subminimal sympathetic doses are maximal parasympathetic doses, as are maximal sympathetic doses. The threshold gradient for parasympathetics does not change, while that of the sympathetics does. This is represented graphically in Chart I.

Another factor which must be taken into consideration is the blood pressure. Bayliss and Starling[35, 36, 37] have shown that anemia causes a cessation of intestinal movements. They found that raising the pressure by

compressing the abdominal aorta caused a diminution in intestinal activity. They concluded also that the primary inhibition following stimulation of the vagus was due in part to the decreased blood supply to the gut. In our experiments the amount of adrenalin producing increased activity did not affect the blood pressure sufficiently to consider it as an important factor (Fig. 6). A rise or fall of but a few mm. of Hg. usually resulted, but never any marked change. So we have not considered the blood pressure as a causative factor.

The adrenalin augmentation does not appear immediately after injecting but after a latent period equal to two to three intestinal waves. This also suggests vagus activity, in view of the fact that usually following vagus stimultaion there is a latent period before contractions begin. This normal action of the intestine has been emphasized by Bayliss and Starling. It would seem to indicate also that adrenalin does not influence the inhibitory fibers to the gut which run in the vagus, since a primary decrease is not noted.

With one exception, the action was of short duration, a fact which indicates adrenalin activity. The usual period of augmentation endured for a time equal to the length of an adrenalin curve.

The possibility that this action might be due to the eserine must be considered. In no case, however, do we find the eserine by itself producing changes either in the heart or intestines in the amount we employed. Physostigmine action usually lasts for a period of a few hours, while this persists for a few minutes at the most.

Large doses of eserine or repeated small doses give rise to marked intestinal activity. Under these conditions adrenalin does not further increase the movements, probably because the eserine action is already maximum.

CONCLUSIONS

1. Eserine lowers the threshold of the vagus to the intestines to electrical stimulation, i.e., sensitizes the peripheral apparatus.

2. In minute doses, adrenalin stimulates the peripheral vagus apparatus and is synergistic with eserine.

3. The vagus threshold is normally lower to adrenalin, but the maximal effect is so much less than that of the sympathetic nerves that the action of the latter predominates unless very small amounts of adrenalin are used.

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THE ELECTROCARDIOGRAM IN CORONARY THROMBOSIS*

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Within recent years, coronary thrombosis has become a recognizable clinical condition, yet the diagnosis in many instances is first made at the postmortem examination.

As the clinical history and physical findings of coronary occlusion are frequently insufficient for a positive diagnosis, a method of greater precision is desirable. For this purpose the electrocardiogram has been used. Several different forms of abnormal curves, considered typical of this cardiac disorder, have been published. A variety of irregularities of rhythm and conduction have been observed which, however, are not peculiar to, or diagnostic of, this disease. The occurrence in this disorder of different types of extrasystoles is not uncommon. In four patients reported by Robinson and Herrmann,[1] they observed ventricular tachycardia. The type of curve considered by many as indicating arborization block was frequently observed in this disease by Herrick.[2] The electrocardiographic change considered diagnostic of coronary thrombosis has been confined mainly to alterations in the T-waves.

Clinical Observations.—In an individual with thrombosis of a coronary artery, Pardee[3] observed most striking alterations in the T-waves of the patient's electrocardiogram. The first tracing, obtained on the fourth day of the patient's illness, showed altered T-waves in Leads I and II. These waves were increased in height and branched directly from the R-wave high up on

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its descending limb. The descending limb of the R-wave was much shorter in Lead III than in Lead II. Fortunately Pardee had the opportunity of obtaining tracings during the succeeding sixteen days and observed a progressive decrease in the size of the T-waves and a steady descent of the R-T branching to isoelectric. In the final stage observed, the T-waves were inverted. The inverted T-waves presented steep straight sides and an acute apical angle. The numerous electrocardiograms from this patient show that no single alteration in the tracings is to be expected from patients suffering with acute coronary occlusion.

It is believed by Kahn[4] that the electrocardiogram shows a characteristic alteration in thrombosis of a coronary artery. This he considers as an initial increase in the height of the T-waves which later become sharply inverted and finally return to isoelectric. The curves which he has published show a moderate increase in the height of the T-waves in Lead I with a branching of the T-waves off from the descending limb of the R-waves. In Leads II and III a deep S-wave is present, the ascending limb of which rises above isoelectric and the T-wave branches directly off from the upper end of this elevated limb. This curve resembles that obtained by Pardee at about the eighth day of his observations on his patient.

In Wearn's[5] series of ten cases of coronary thrombosis, there was some alteration in the T-waves of each electrocardiogram. The change in some cases was present in Lead I, in more cases it was present in Leads I and II. In a few of the tracings the T-waves were inverted, while in others they were isoelectric or diphasic. In only one record did he note the high branching of the T-wave directly from the descending limb of the R-wave and then in only the second lead where it disappeared within twenty-four hours. It was Wearn's belief that there was no form of the electrocardiogram characteristic of coronary thrombosis.

In a recent article, Pardee[6] restates his belief in the characteristic alteration of the T-wave of the electrocardiogram by coronary thrombosis. He has found that the change occurs in one or more, but usually only in one lead. He describes what he considers the typical change as a downward, sharply peaked T-wave immediately preceded by an upwardly convex S-T or R-T interval. If this condition is present in Lead III alone, he considers it of no significance, but if the T-wave is also inverted in Lead II, even though this latter is not preceded by this upwardly convex R-T interval, he considers it of diagnostic importance.

The occurrence of ventricular tachycardia with coronary thrombosis was the point dwelt upon by Robinson and Herrmann[1] in their report of four cases. The electrocardiogram of their Case 1 obtained after the cessation of the tachycardia does show a branching of the T-waves directly off from the descending limb of the R-waves in Lead I. This condition is even more pronounced in Leads I and II of the electrocardiogram taken in their Case 2 after the tachycardia had stopped. They state that Cases 1 and 2 showed the most evidence of obstruction in the coronary arteries.

In the nine cases of coronary thrombosis recently reported by Willius,[11] his published curves show branching of the T-waves directly off from the R- or S-waves with absence of the isoelectric R-T or S-T interval in four cases and, as he states, this is such a definite alteration that its presence should always direct attention to the possible existence of acute coronary obstruction. The inversion of the T-waves in one or all leads was present in eight of his nine cases.

In considering the electrocardiograms of coronary thrombosis, it is necessary that caution be followed and that the diagnosis be confirmed as far as possible by our clinical methods or by autopsy. All cases where doubt may arise as to the accuracy of the diagnosis should be excluded from consideration. We present the following cases, the diagnosis having been made either on histories and physical findings generally considered as typical of this

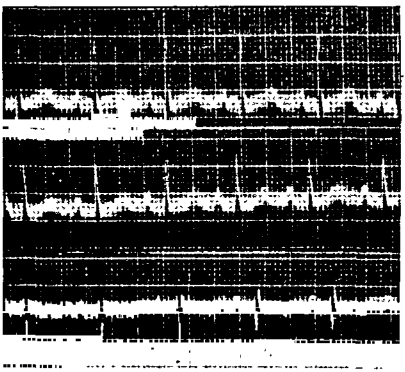

Fig. 1.—Electrocardiogram taken eight months before the attack of severe heart symptoms.

condition, or on autopsy. The electrocardiographic changes in these patients are those which we have come to consider as strongly suggesting this disease. One of our patients may possibly be placed in the second group of Herrick's classification as he died within a few hours after the probable onset; the others belong in his fourth group, the patients having recovered and regained a slight amount of cardiac efficiency.

CASE REPORTS

Case 1.—An insurance salesman, aged fifty-one. This man was admitted to the hospital complaining of a recent attack of severe agonizing precordial pain which lasted several hours. He also complained of dyspnea and edema.

History.—His only past illness had been typhoid fever. He was examined by his home physician in May, 1923, at which time his heart was found to be enlarged and his exercise tolerance poor. He had no edema but did complain of dyspnea. His blood pressure was 150 systolic and 90 diastolic and his heart rate was 100. He improved by rest in bed. His

present illness started in December, 1923, as a sudden, severe, persistent and agonizing substernal pain, not relieved by nitrites.

Examination.—He was a man of large frame and moderately obese. No dyspnea was observed. The skin had a sallow, pasty color. The chest was barrel-shaped, the lungs being moderately emphysematous but showed no congestion at their bases. The left border of cardiac dullness extended 15 cm. from the midsternal line in the fifth interspace. There was a gallop rhythm, the heart sounds being very distant. The radial arteries showed moderate thickening. The blood pressure was 125 systolic and 100 diastolic and there was edema of both ankles. Dyspnea could be produced on the slightest exertion and the vital capacity of the lungs was 2,200 c.c. No friction rub was heard.

E. K. G.—An electrocardiogram taken by his home physician in May, 1923, is reproduced in Fig. 1 and shows no definite abnormalities. In Fig. 2 is a second curve taken in December, 1923, eighteen days after his attack of severe precordial pain. This tracing shows inversion of the T-waves in all leads.

Course.—This patient continued in a state of severe myocardial insufficiency and died in May, 1924.

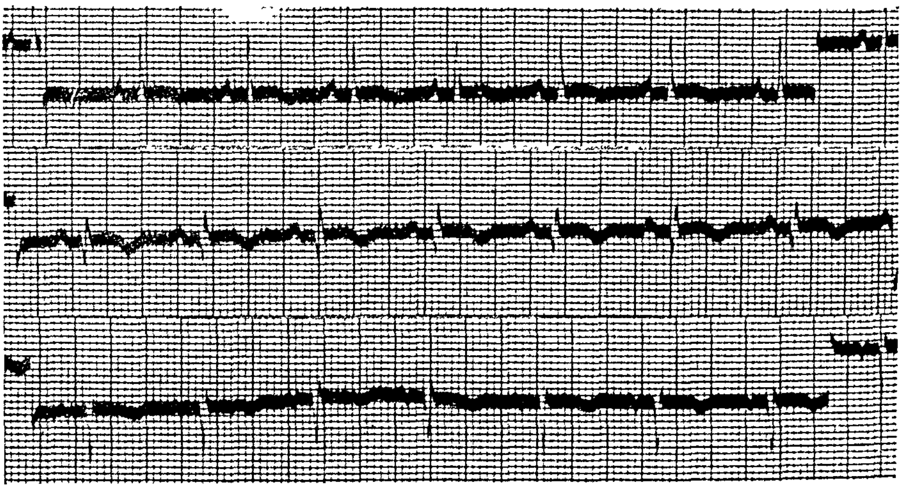

Fig. 2.—Electrocardiogram taken eighteen days after onset of heart symptoms.

CASE 2.—An elderly man, with no occupation, aged sixty-five. He first entered the hospital on July 30, 1924, complaining of an attack of severe agonizing substernal pain which had lasted several hours and was not relieved by any medication. He also was very weak and had dyspnea, edema, gastric distress and flatulence.

History.—He had severe pneumonia at the age of twenty-five, but otherwise had been unusually healthy up to his present illness.

The present trouble had started on July 24, 1924, when suddenly and without provocation he was seized by a severe, cutting, almost unbearable, pain in the substernal region. This pain lasted several hours and was accompanied by dyspnea and orthopnea. He further stated that with the attack, his heart "fluttered" and his lips were purple.

Examination made on August 2, 1924, showed a short, obese man who was suffering from severe dyspnea and orthopnea. His lips and extremities were extremely cyanosed. The apex impulse of the heart was not seen or felt. The left cardiac border was 14 cm. from the midsternal line in the fifth interspace. The heart rhythm was regular, the sounds distant and of very poor quality. There were no murmurs. The blood pressure was 110 systolic and 70 diastolic. The heart rate was 100. The lungs were emphysematous with moisture at both bases. The liver was tender, its edge being felt 2 cm. below the costal margin. The radial arteries were thickened and edema was present in the legs. No elevation of temperature was noted.

E. K. G.—The first tracing (Fig. 3) taken August 5, 1924, showed a branching of the T-waves off from the descending limb of the R-wave in Leads I and II, there being no isoelectric R-T interval. On February 20, 1925, a second curve (Fig. 4) showed a flattened, almost isoelectric T-wave in Lead I, the branching of the T-waves directly off from the R-waves in Leads I and II having disappeared.

Course.—Up to February, 1925, the patient continued to show dyspnea, which increased on slight exertion, the liver remained enlarged and edema persisted in the legs but there had been no recurrence of the chest pain.

CASE 3.—Attorney, aged fifty-nine. He entered the hospital on February 5, 1925; complaining of dyspnea, orthopnea and edema.

History.—He had had rheumatism of unknown type and gonorrhea as a boy. Symptoms of heart disease had first appeared in March, 1923. He had been previously admitted to the hospital in May, 1924, complaining of cough and dyspnea. Treatment afforded him considerable relief. These symptoms recurred with greater severity but a few weeks before his last admission, with also palpitation, nausea and vomiting. There was no complaint of pain.

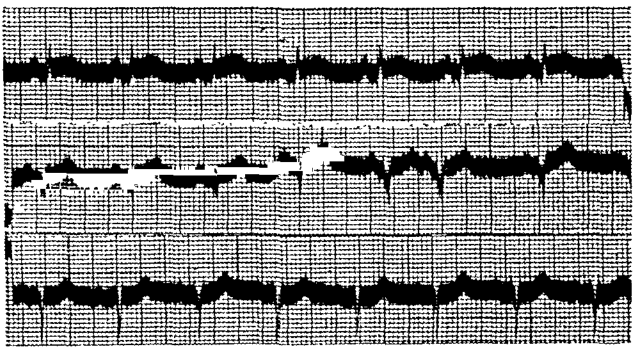

Fig. 3.—Electrocardiogram taken a few days after onset of heart symptoms.

Examination.—He was moderately obese. The pupillary reaction to light and tendon reflexes all gave normal responses. Dyspnea and cyanosis were very evident with increased pulsation in the great vessels of the neck. The apex impulse could not be localized. The left border of cardiac dullness was 15 cm. from the midsternal line in the fifth interspace. The heart rhythm was regular, with frequent extrasystoles, the second aortic sound being accentuated. A blowing systolic murmur was heard at the apex, replacing the first sound. No friction rub was heard. The blood pressure was 150 systolic, 95 diastolic, with palpable thickening of the radial arteries. Edema was present at both lung bases and pitting edema in both lower extremities. The liver edge was felt 4 cm. below the costal margin. The blood Wassermann reaction was negative.

E. K. G.—The one electrocardiagram was obtained eighteen hours before death. This curve (Fig. 5) showed frequent extrasystoles, a broadened QRS complex and apical notching of the maximum deflections in Leads II and III. The most striking change was the loss of the R-T interval in Lead I with the branching of the T-wave directly from the descending limb of the R-wave. In Lead III, the T-wave likewise branched directly off from the S-wave definitely below the isoelectric line.

Course.—This patient died a few days after his admission to the hospital.

Autopsy (By Dr. C. C. Garber):

Heart.—The coronary arteries of the heart were injected with an opaque mass and x-ray pictures were taken. This procedure did not give us any evidence of artery obstruction. The heart weighed 635 gm. The left ventricular wall at the apex measured 10 mm. in thickness. The right ventricular wall was 6 mm. and the left 18 mm. in thickness while the posterior wall of the left ventricle measured only 7 mm. in thickness. The valves were all intact and not unusual. The endocardium was smooth and glistening. The posterior papillary muscles were smaller than usual. The cut myocardium showed a reddish brown appearance. In the region of the apex, the muscle was thinned out and grey streaks of connective tissue were present. The posterior wall of the left ventricle in the distribution of the circumflex branch of the left coronary artery was thinned out. The section of the muscle wall in this area consisted almost entirely of connective tissue. The coronary orifices were not unusual but there were a few areas of yellow intimal thickening about them in the sinuses of Valsalva. Occasional patches of intimal thickening were noticed along the coronary vessels. The left coronary artery near its origin and also the circumflex branch were narrowed, the latter so that a small prcbe could not be passed into it.

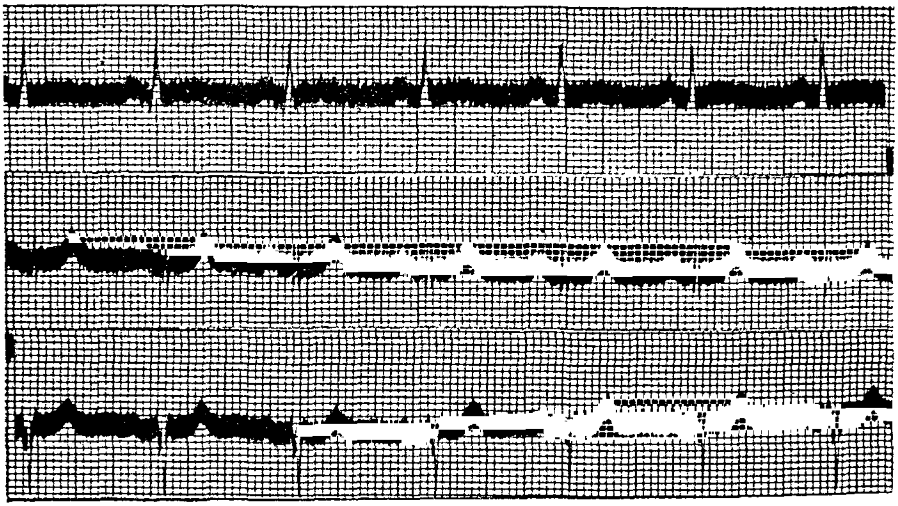

Fig. 4.—Electrocardiogram taken about seven months after attack of severe heart symptoms.

Microscopic:

Heart.—(Near the apex of the left ventricle.)

The muscle striations were poorly preserved. There was a moderate amount of diffuse scarring with replacement of atrophic muscle cells by fibrous tissue. In some of the scars there were numerous polymorphonuclears and a few wandering cells. A small area of necrosis, infiltrated by polymorphonuclears was seen. A vein in the epicardium showed a small thrombus occupying about one-third of its lumen. No bacteria were seen in the special stained sections.

(Thinned out portion of left ventricle.)

The endocardium and epicardium were slightly thickened by connective tissue. The muscle cells showed brown atrophy. In many areas the muscle cells were undergoing degeneration and atrophy, some being replaced by connective tissue. There were several areas of hemorrhage. Several capillaries contained the injection mass but several large arteries were empty.

Aorta.—The intima was thickened and partially hyalinized, the surface layers were also infiltrated by a small number of wandering cells. There was some calcification and no perivascular round-cell infiltration.

Diagnosis.—Arteriosclerosis, partial occlusion of the circumflex branch of the left coronary artery with localized atrophy and scarring of the myocardium; thrombosis of a smaller branch with infarction; myocardial degeneration.

Experimental Observations.—In 1909 Eppinger and Rothberger[7] observed that the injection of silver nitrate solution deep into the wall of the dog's heart, destroying the left ventricular muscle, caused a peculiar and changing alteration in the electrocardiogram. This change consisted of an elevation of the T-waves which branched directly off from the descending limb of the R-wave. This T-wave gradually changed, the R-T branch descending lower and lower till it approached isoelectric with finally the reappearance of the isoelectric R-T interval.

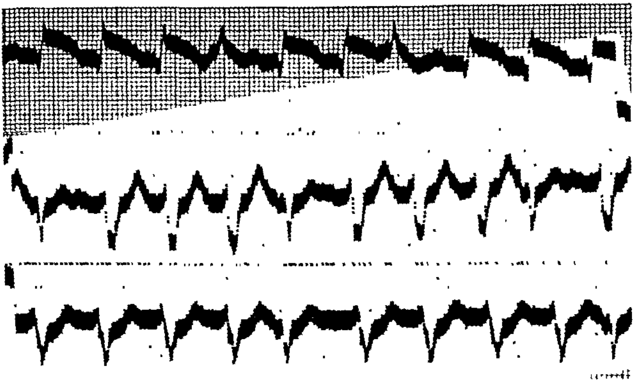

Fig. ... and apparently but a short ... and fatal heart attack.

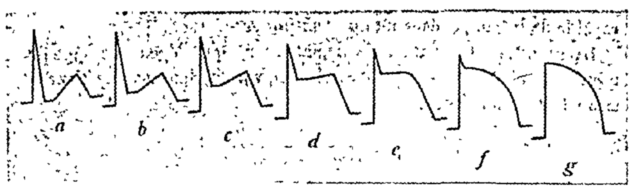

Fig. 6.—Samajloff's curves showing the action of the demarcation current produced by injury at the apex of the heart on the electrocardiogram. The figure (*g*) shows the effect of maximum apical negativity due to muscle injury. The gradual decreasing of the results of apical injury on the electrocardiogram is shown by the changes back to the normal form (*a*).

In 1918 Smith,[8] operating on dogs, ligated branches of the coronary arteries. The electrocardiograms from a large percentage of his dogs showed the T-waves branching off from the descending limb of the R-waves. The T-waves gradually approached isoelectric and finally became inverted. He repeated these experiments in 1920[9] and found that change in the T-wave was greatest in those dogs where the blood supply to the apex of the heart was most disturbed. Accordingly, he obtained the greatest changes where two or more branches of the ramus descendens and the circumflex sinistra which

supply the apical area were ligated. The ligation of arterial branches which supplied the right ventricle did not produce these changes in the electrocardiogram.

For years it has been known that the cutting of one end of a muscle bundle causes a difference of potential to arise between the cut end and the surface of the remaining uninjured portion. The injured end becomes electronegative, the surface of the uninjured portion being electropositive. Under

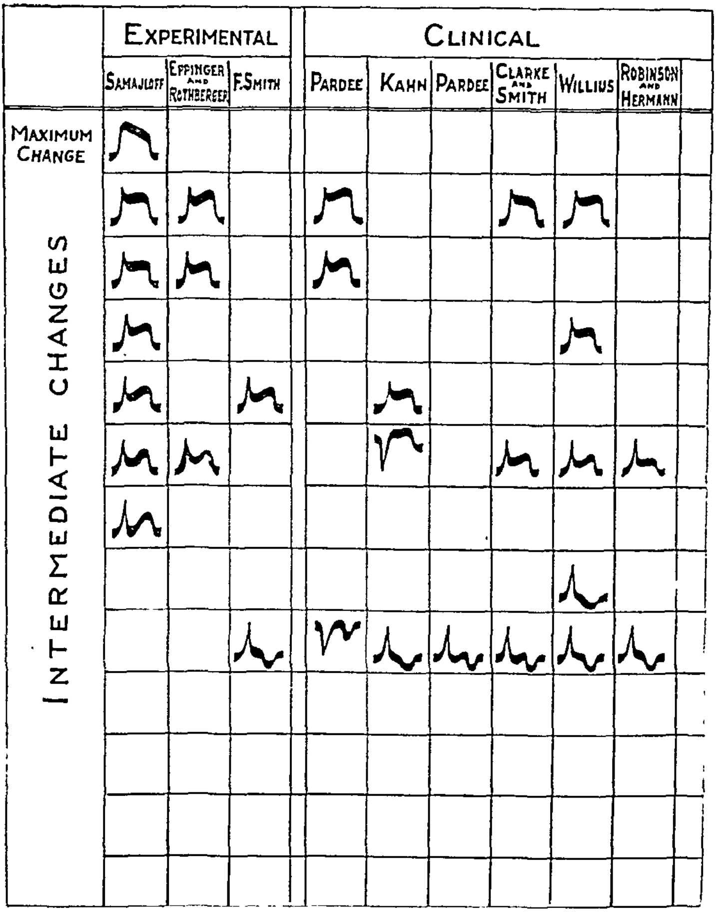

Fig. 7.—Comparison as to form of the transitional curves experimentally produced by injury to the ventricular musculature with the published curves of cases of coronary occlusion.

favorable circumstances, a current will flow from the positive portion of the muscle through a conducting medium to the injured or electronegative end of the muscle. This current, though very slight, can be detected and measured by the finer methods of precision such as by the electrocardiograph.

This physiologic fact has been extended by Samajloff[10] in working with frogs' hearts. He applied his electrodes directly on the heart at the apex and base. He found that pinching or cutting the heart muscle at its apex caused the T-wave of the electrocardiogram to be elevated and branch directly from the R-waves, the duration of the altered curve or its progressive

return to its previous normal type depending on the depth of the muscular cut, and on the age of the heart muscle.

In the frog's heart with apical injury, this peculiar alteration in the T-waves of the electrocardiogram was found by Samajloff to show a recession within five minutes, the return to normal being completed within one hour. The current set up by the difference of potential between the dead and living portions of the muscle fibers, ceased as soon as the injured fibers were entirely dead. In animals, the monophasic current derived from putting two electrodes on the heart, one on the injured and one on the uninjured surface, is temporary. The curves gradually change from the monophasic to the usual type obtained in direct leads. The progressive change in the electrocardiogram due to the interference of the demarcation current as observed by Samajloff are shown in Fig. 6.

Comparison of Clinical and Experimental Curves.—In reviewing the electrocardiograms from our own patients of coronary thrombosis, those of published cases and the mentioned experimental records, we noticed two striking similarities. The first was the same cyclic transition or variation of the tracings as time progressed and secondly the same elevation of the T-waves, branching directly off from the R- or S-wave, occurring in the true cases of coronary thrombosis and those tracings obtained by various experimental methods, all of which have included injury to the ventricular muscle. The chief difference between the clinical and experimental electrocardiograms is that the first were obtained by indirect leads whereas the experimental tracings were obtained by leads placed directly on the heart.

In Fig. 7, the experimental curves produced by the demarcation current at the apex of the frog's heart, also by tying off the coronary arteries of a dog and by injecting silver-nitrate solution into the ventricular muscle of the dog's heart are compared with those obtained from the human heart after obstruction to the coronary arteries due to disease.

This chart readily shows that the various examples of electrocardiograms which have been considered as indicating coronary thrombosis in the human subject can all find their close counterpart in the series of curves showing the effect of experimental injury to the muscle of the left ventricle.

The experimental findings of Smith by coronary artery ligation and of Eppinger and Rothberger by injecting silver-nitrate solution into the deep layers of the left ventricular muscle likewise find their counterpart in Samajloff's curve of the demarcation current produced by injury to the apex of the frog's heart. This similarity is more striking when one recalls that Smith could not produce this electrocardiographic change by obstructing those coronary branches which supply the right ventricular chamber.

This peculiar abnormality of the electrocardiogram has been observed in acute rheumatic fever by Cohn and Swift.[12] They thought it was not characteristic of the nature of the injury but rather the result of injury involving a given area. They inferred that its occurrence in rheumatic fever was due to Aschoff nodules or perhaps ischemic areas consequent upon blood vessel involvement causing injury to the muscle with subsequent electrical

disturbance. Their explanation of ischemic areas is the same as arises in coronary vessel obstruction and the "given area" which they considered involved seems on clinical and experimental grounds to be in the left ventricle and at or near the apex of the heart.

The forms of the electrocardiograms which we have presented as evidences of coronary obstruction evidently occur shortly after the injury and with the passage of time, the electrocardiogram is replaced by one not necessarily characteristic.

SUMMARY

The diagnosis of coronary thrombosis is frequently doubtful and the electrocardiograph has been shown to be of decided assistance. Numerous cardiac irregularities have been noted which are not characteristic.

The alteration of the electrocardiogram characteristic for occlusion has generally been confined to changes in the T-waves.

No definite alteration of the T-waves in coronary thrombosis has been uniformly accepted by the many writers as characteristic.

We have reported three cases with electrocardiograms, one having been confirmed by autopsy.

In experimental investigations, where injury was produced to the ventricular muscle, Eppinger and Rothberger, Smith, and Samajloff found that the T-waves of the electrocardiogram were elevated and branched directly from the R- or S-wave. They further noted that with time this elevation of the T-wave, descended to isoelectric with a reappearance of the isoelectric R-T or S-T interval.

In Samajloff's experiments he found the same change in the T-waves of the electrocardiogram due to the interference of the demarcation current between the injured area of negativity and the positively charged surface of the uninjured muscle.

The published clinical curves all find their counterpart in the series of tracings obtained by these various experimenters. The experimental curves were taken by leads directly on the heart, whereas the clinical records are from arm and leg leads.

This change in the electrocardiogram has been observed in acute rheumatic infections by Cohn and Swift. They consider it due to possible blood vessel involvement causing muscular injury with subsequent electrical disturbance.

A characteristic electrocardiographic change appears when the coronary obstruction causes injury to the left ventricle, but is not as constant when the right ventricle is involved.

The similarity of the electrocardiograms obtained by experimental injury to the ventricular muscle through various methods with electrocardiograms from clinical examples of coronary thrombosis, suggests that the acute muscular insult in both instances causes similar electrical disturbances which produce a characteristic change in the T-waves of the curves. For clinical

purposes this change consisting of elevation of the T-waves which branch directly from the R or S limb may be considered as strong presumptive evidence of coronary occlusion.

If this peculiar change is typical for coronary thrombosis in the human subject it is essentially more or less transient and soon is replaced by changes not characteristic.

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THE TOXICITIES OF LOCAL ANESTHETICS

WITH SPECIAL REFERENCE TO APOTHESINE*

BY HERBERT C. HAMILTON, DETROIT, MICH.

THE increasing use of local anesthesia has made the physician and surgeon more and more discriminating in his selection of the agent. The discovery and clinical use of cocaine by means of which minor operations could be carried out without general anesthesia is regarded as one of the epochs in the field of therapeutics. Were it not for the after effects of this valuable agent no substitute would ever have been sought. Its habit-forming effects leading to drug addiction have to a very great extent discredited cocaine. Its toxicity, while not to be disregarded, is ordinarily a less serious objection.

The long list of substitutes—eucaine, β-eucaine, holocaine, stovaine, alypin, novocaine—to mention only the outstanding ones—indicates the almost feverish interest shown in finding a satisfactory substitute for cocaine.

Of the more recently proposed local anesthetics, apothesine and butyn have been used enough to give a fair line on their availability, while isocaine, in which a propyl radical replaces the ethyl group of novocaine, is the latest one for which any data are available.

With this increasing number of local anesthetics more information is needed in order that the advantages and disadvantages of each may be fully known. Unfortunately, however, the results of tests vary greatly, indicating that the methods used and the results obtained are capable of widely different interpretation. For example, Braun[1] says, "By doubling the dose of novocaine so as to make it as effective as cocaine—novocaine has become the ideal anesthetic for injection into the tissues and has made the use of cocaine unnecessary." This was later practically verified by Closson,[2] except that, for nerve blocking, he regarded it as better than cocaine. Sollmann[3] gave novocaine a nerve-blocking value equal to cocaine but showed its value for the cornea to be one-sixteenth, for terminal anesthesia, as measured on the frog's skin, one-fourth that of cocaine. Schmitz and Loevenhart[4] showed procaine (novocaine) to be six times as effective as cocaine for nerve blocking.

Using apothesine in comparison with cocaine, Sollmann[5] at first reported it one-eighth by terminal anesthesia (intracutaneous injection into human subjects). He later revised his opinion as published by the Council on Pharmacy and Chemistry,[6] saying the value of apothesine is not better than one-half that of cocaine. By the frog skin method, Heinekamp[7] reported apothesine one-sixth as valuable as cocaine and novocaine of no value.

That competent pharmacologists should get such widely different results is evidence that accurate methods are not available by which to measure the efficiency of local anesthetics.

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Even the toxicity is variously reported. Closson[2] found cocaine eight times as toxic as novocaine, using guinea pigs subcutaneously. Hatcher and Eggleston reported a ratio of 3 to 1 by rapid intravenous injection of one large dose into cats, the actual M. L. D. being, cocaine 0.015; novocaine 0.045. Meeker and Frazer[8] found that for novocaine 0.065 was required to kill a majority of the rabbits used, by intravenous administration.

It would appear that further data on the relative toxicities of apothesine and cocaine will tend to remove some of the uncertainties regarding this important factor in local anesthesia.

When comparing drugs of similar character for efficiency and toxicity, these two effects are usually parallel. In some cases, however, such parallelism does not follow because side chains of organic compounds may vary in the intensity of the one or other of these two effects.

Apothesine is a local anesthetic one-fifth as toxic as cocaine hydrochloride[9] and equal to it for nerve blocking and terminal anesthesia.

For mucous membrane anesthesia it is one-third as effective.[10]

These results were obtained by known methods.

The toxicity data were obtained in the usual way on guinea pigs subcutaneously administered. The efficiency data for nerve blocking were obtained by action on the isolated sciatic nerve of the frog. The nerve-muscle preparation was immersed in the solution and its degree of anesthesia deduced from its response or lack of response to electrical stimulation; terminal anesthesia was tested by intracutaneous administration to four human subjects; and mucous membrane anesthesia, the Gradenwitz method,[11] by dipping one leg of a frog into the solution. The frog has its brain, medulla and heart removed. The leg is dipped into the solution for one minute, is removed and washed, and at intervals both feet are dipped into weak acid. Comparing the response to this irritation one can readily select an equally effective dilution for both the cocaine and the substance in question.

If this method is applied with almost infinite care and attention to details, the results can be regarded as trustworthy but of limited value. Heinekamp,[7] however, seems to have omitted some of the essential elements of the method, since he himself decided that they were valueless.

Ordinarily when the effective dose of a substance is far below the lethal or even mildly toxic dose, the toxicity of that substance may be disregarded. In the case of local anesthesia, however, fatalities have occurred[12] which make it necessary to investigate the causes and to consider relative advantages, having due regard for the factors of efficiency and safety.

Careful investigations of the deaths from local anesthetics reveal the fact that carelessness is often responsible[8]—carelessness in administration and even in the strength of solution. Aside from these the most important factor influencing the acute intoxication sometimes observed is the rate of absorption into the blood stream[8] (p. 389).

This fact is responsible for selecting the intravenous administration of the drug as a means of determining its toxicity. Since the reported cases of fatal poisoning are, however, so rare[12] (p. 437), and it is in most cases an

even chance that carelessness of some kind was responsible, the toxicity by this method is misleading.

I have recently compared apothesine with cocaine, not only by the intravenous administration into cats, but also by subcutaneous injection, using cats, mice and guinea pigs, and by intraperitoneal injection into mice.

The M. L. D. for mice is the same by both methods:	
Cocaine	150 mg. per kg.
Apothesine	700 " " "
ratio:	nearly 5 to 1.
The M. L. D. for guinea pigs subcutaneously:	
Cocaine	50 mg. per kg.
Apothesine	250 " " "
ratio:	5 to 1.
The M. L. D. intravenously to cats:	
Cocaine	10 mg. per kg.
Apothesine	25 " " "
ratio:	2.5 to 1.

The M. L. D. subcutaneously to cats cannot be compared on account of the rapid elimination of the apothesine. No death occurred with any dose administered. The M. L. D. of cocaine and maximum of apothesine administered were:

Cocaine	60 mg. per kg.
Apothesine	800 " " "

This is explainable by the fact that apothesine is rapidly eliminated at a rate which almost equals absorption. Eggleston and Hatcher[12] bring this point out clearly, showing that nine and one-half times the intravenous fatal dose of apothesine was administered into cats subcutaneously without producing convulsions. Further, that doses just sublethal can be administered intravenously at twenty-minute intervals almost indefinitely.

This is eloquent testimony to its safety in anesthesia.

There is, of course, always a question as to how much dependence may safely be placed upon toxicity tests carried out on animals. It is not logical to assume any relationship between the M. L. D. for any animal and that for man. The difference in susceptibility of different animals—dogs, cats, rats, pigs—indicates that similar or greater differences would be found in the lethal doses for any species and for man.

This seems to be especially true for a local anesthetic, if one accepts the occasional deaths as due to no other factor than to the anesthetic itself. Data seem to indicate that in most cases carelessness and in some cases idiosyncrasy is responsible. On occasions it is highly probable that it is a coincidence with some other responsible poisoning.

The real value of toxicity tests does not lie in the absolute M. L. D. but in the ratio of the M. L. D. of the substance in question to that of a similar substance whose clinical behavior is known.

This is a point often overlooked by pharmacologists, leading to wrong conclusions. No dose for any species of animal can safely be transferred to

man. Differences in the method of administration and susceptibility, to say nothing of the infinitely great difference in order of intelligence, make the use of the cat as the animal most nearly approaching man, a ridiculous assumption.

These data show, therefore, that apothesine is relatively only one-fifth as toxic as cocaine, except when it enters the blood stream in considerable quantity, an eventuality which can scarcely be regarded as having any bearing on the common use of a local anesthetic except in close proximity to a large blood vessel.

The purpose of using adrenalin with a local anesthetic is primarily to localize the effect by preventing absorption materially beyond the area injected, taking advantage of the well-known constricting action of adrenalin. There is, in addition to this, the strongly stimulating effect it has on the heart, an effect exactly the reverse of that of cocaine or apothesine. While this action is only slight from a subcutaneous administration, intravenously the action is prompt; it very definitely tends to offset the depressant effect of the anesthetic.[13]

There is, therefore, a double logic in associating these two powerful substances.

The extensive and satisfactory use of apothesine in anesthesia,[14] the few cases of poisoning reported, and the laboratory data showing its efficiency, low toxicity and prompt elimination in comparison with cocaine, indicate that apothesine more nearly duplicates cocaine for terminal anesthesia and nerve blocking than any other local anesthetic.[15]

TOXICITY

METHOD	SUBCUTANEOUS							INTRAVENOUS					
Reference	(9)	(2)	(4)	(14)	(16)	(17)	Average	(12)	(16)	(14)	(9)	(16)	Average
Cocaine	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Novocaine	0.12	0.12	0.14		0.2		0.15	0.35	0.26				0.3
Apothesine	0.2						0.2	0.75			0.4		0.57
Butyn			1.5	2.5		3.6	2.6			1.0		1.0	1.0
Isocaine			0.3				0.3						

EFFICIENCY

METHOD	NERVE BLOCKING					TERMINAL				MUCOUS MEMBRANE		CORNEA	
Reference	(2)	(1)	(4)	(3)	(9)	(2)	(3)	(5)	(9)	(7)	(9)	(4)	(3)
Cocaine	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Novocaine	1.0	0.5	6.0	1.0	1.0	1.0	1.0	1.0	0.33		0.33		0.6
Apothesine					1.0		0.12	0.5	0.33	0.16	0.33		0.12
Butyn			3.0							1.0		0.9	
Isocaine			6.0									1.4	

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LABORATORY METHODS

AN ACCURATE METHOD FOR THE ESTIMATION OF UREA IN BLOOD AND URINE BY DIRECT NESSLERIZATION*

By Joseph H. Roe, Ph.D., and Oliver J. Irish, M.S., Washington, D. C.

VARIOUS procedures[1, 2, 3] are in use at the present time for estimating the urea in protein-free blood filtrates, or untreated urine mixtures, by enzymic hydrolysis and direct nesslerization of the ammonia in the hydrolysate. These methods are all open to the serious objection that more or less turbidity develops in the nesslerized mixture, and turbid mixtures cannot be read colorimetrically with any degree of accuracy.

The turbidity obtained when nesslerizing directly urines, or tungstic acid blood filtrates, which have received urease treatment, is due to the precipitation of the mercury in the Nessler's reagent by traces of proteins, and other organic matter, in both the urease solution and the substrate. The solution of the problem of producing satisfactory colors when nesslerizing such mixtures is the removal of mercury precipitants. After an investigation of many clarifying procedures, the authors have found that the turbidity-producing substances can be removed most conveniently and satisfactorily by treatment with calcium phosphate in an alkaline medium. This procedure consists of alkalinizing the mixture, adding a little calcium nitrate, which precipitates the phosphate previously added as a buffer for the urease digestion, mixing thoroughly, and centrifuging The calcium phosphate adsorbs the objectionable organic matter, and the clear supernatant solution obtained after centrifuging is decanted and nesslerized. The color obtained in a solution filtered free from mercury precipitants by this procedure compares excellently with that produced by a pure ammonium salt solution when nesslerized.

PROCEDURE FOR BLOOD

Pipette 2 c.c. of blood into a small Erlenmeyer flask and add 1 c.c. of potent urease solution and 2 drops of Folin's buffer phosphate mixture. Cork and place at 50° C. for fifteen minutes. After enzymic digestion, the blood is treated to produce a protein-free filtrate according to the regular Folin-Wu procedure. Add 13 c.c. of distilled water, 2 c.c. of sodium tungstate, and 2 c.c. of two-thirds normal sulphuric acid; mix thoroughly, allow to stand for two or three minutes until the typical "chocolate brown" color appears, then filter through an ammonia-free 9 cm. folded filter paper. (Fil-

*From the Department of Chemistry George Washington University Medical School. Received for publication, April 28, 1926.

ter paper around 9 cm. in size is desirable to get the most filtrate.) Place 10 c.c. of the tungstic acid filtrate in a 15 c.c. conical centrifuge tube, add 0.5 c.c. of 20 per cent sodium hydroxide, 1 drop of buffer phosphate mixture (to insure precipitation of calcium), 2 drops of 10 per cent calcium nitrate, and stir the precipitated calcium phosphate with a glass stirring rod until it is distributed uniformly through the mixture. Centrifuge for two minutes, then decant the supernatant liquid into a 25 c.c. graduated cylinder. Wash down the sides of the centrifuge tube with 2 c.c. of 1 per cent sodium hydroxide, and again mix uniformly the calcium phosphate by breaking up the mat in the bottom of the tube and stirring with a glass rod. Centrifuge for two minutes, then decant the supernatant fluid into the original mixture. Add 3 c.c. of Nessler's reagent, and make up to 15 c.c. with distilled water. This will be the correct volume for color comparison if the blood has a normal urea content. If an increased urea is encountered, the mixture is diluted to a volume that will approximately match the standard by adding distilled water and Nessler's reagent, the amount of the latter introduced being one-fifth the final volume of the mixture.

Prepare a nesslerized standard nitrogen solution as follows: In a 100 c.c. glass-stoppered graduated cylinder place 5 c.c. of standard ammonium sulphate solution containing 1 mg. of nitrogen; add distilled water to the 50 c.c. mark, Nessler's reagent to the 70 c.c. mark, then distilled water to 100 c.c., and mix thoroughly. Compare in a colorimeter in the usual manner.

CALCULATION

$$\frac{\text{Reading Standard}}{\text{Reading Unknown}} \times 1 \times \frac{\text{Dilution Unknown}}{100} \times 100 = \text{Mg. Urea N per 100 c.c. Blood.}$$

The step where the centrifuge tube is washed with 2 c.c. of 1 per cent sodium hydroxide may be omitted in clinical work without appreciable loss of accuracy by introducing a correction factor for the amount of ammonia in the fluid left in the tube after the first decantation. We have found that the average amount of water left in a cleanly draining tube after decantation is around 0.5 c.c. This is about one-twentieth of the solution being analyzed, and will therefore require a 5 per cent correction factor. This correction is conveniently introduced by substituting 105 for 100, the last factor in the above formula for calculation.

PROCEDURE FOR URINE

Transfer 5 c.c. of urine to a 50 c.c. volumetric flask, make up to the mark with distilled water, and mix thoroughly. Pipette 1 c.c. of this mixture into a 15 c.c. conical centrifuge tube, add 1 c.c. of potent urease solution, 2 drops of Folin's buffer phosphate mixture, and place the tube in a water-bath at 50° C. for fifteen minutes. At the completion of the enzymic digestion remove the tube and add 8 c.c. of ammonia-free water, 1 c.c. of 10 per cent sodium hydroxide, and 2 drops of 10 per cent calcium nitrate solution. Stir the mixture with a glass stirring rod until the calcium phosphate precipitate is uniformly distributed, then centrifuge for two minutes. After centrifugation,

decant the clear supernatant liquid into a 100 c.c. glass-stoppered graduated cylinder. To remove the ammonia adherent to the sides of the centrifuge tube add 10 c.c. of 1 per cent sodium hydroxide, break up the mat of calcium phosphate in the bottom of the tube with a glass stirring rod, centrifuge as before, and decant the supernatant washings into the original mixture. Add distilled water to the 40 c.c. mark, then 10 c.c. of Nessler's reagent, and mix thoroughly. If the color obtained is too deep for favorable comparison, the mixture is diluted to a desirable volume by adding more distilled water and Nessler's reagent, always introducing into the solution an amount of Nessler's reagent equal to one-fifth the final volume. Prepare a standard as directed above under *Procedure for Blood,* and compare in a colorimeter in the usual manner.

CALCULATION

$$\frac{\text{Reading Standard}}{\text{Reading Unknown}} \times 0.001 \times \frac{\text{Dilution Unknown}}{100} \times \frac{\text{24 hr. Volume in c.c.}}{0.1} = \text{Grams Urea N} + \text{Ammonia N per 24-hr. sample of Urine.}$$

To obtain Urea N, subtract Ammonia N determined separately by the permutit or other methods.

EXPERIMENTAL AND DISCUSSION

This procedure was checked against the Myers[4] urease and aeration method for urea estimation upon pure urea solutions and upon numerous blood and urine samples. Twenty-four samples of a pure urea solution of

TABLE I

SAMPLE NO.	AERATION METHOD GRAMS UREA N PER 100 C.C. URINE	AUTHORS' METHOD GRAMS UREA N PER 100 C.C. URINE
1	0.55	0.55
2	0.87	1.03
3	1.07	1.13
4	1.20	1.54
5	1.33	1.33
6	1.25	1.25
7	1.21	1.35
8	1.57	1.80

TABLE II

SAMPLE NO.	AERATION METHOD MG. UREA N PER 100 C.C. BLOOD	AUTHORS' METHOD MG. UREA N PER 100 C.C. BLOOD
1	10.5	10.5
2	12.8	13.5
3	12.8	12.7
4	13.6	14.8
5	15.2	14.7
6	32.7	35.7
7	47.5	48.0
8	69.0	68.5
9	128.2	127.5
10	222.0	235.0

This hydrogen electrode is very easily made and requires nothing not ordinarily found in a chemical or biologic laboratory. In its preparation either a simple adapter, such as is commonly used in ordinary distillations, or a short piece of pyrex glass tubing about 15 mm. in diameter is used.

Hydrogen from a generator enters at *A*. The gas rises through the liquid saturating the liquid as it passes through and carrying out the air with it through the holes at *B*. After two to four minutes of gentle bubbling, the tube *C* is pulled up through the rubber stopper so that the holes at *B* become closed, leaving an atmosphere of hydrogen over the liquid. The platinum blacked electrode is now adjusted by means of the glass rod *D*, so that half of the platinum is immersed in the solution. The stopper *E* is now removed and the tip of the arm of the standard calomel cell is immersed slightly below the surface of the liquid in the arm *F* of the hydrogen electrode. In the manipulation care must be taken not to tip the hydrogen electrode about too much or air bubbles will enter and break the capillary column of liquid. This, however, does not destroy the determination provided the column can be reunited immediately.

SUMMARY

1. A modified form of hydrogen electrode requiring less than 1 c.c. of liquid for examination is described. The liquid may be clear, colored, or turbid.

2. The electrode is especially adapted to use with biochemical solutions.

3. The time required to saturate the solution with hydrogen and to bring the electrode to equilibrium is less than five minutes.

Thanks are due to the laboratory staff of the Hermann Hospital for the blood samples used in this investigation.

A MODIFICATION OF GOODPASTURE'S TECHNIC FOR THE PEROXIDASE REACTION IN BLOOD SMEARS*

BY DEAN N. BEACOM, M.D., DENVER, COLORADO

IN 1919 Goodpasture[1] described a stain which is used to demonstrate the peroxidase reaction within certain leucocytes. This is a great aid in differentiating leucemias of the lymphatic and the myelogenous types.

The stain which he prepared has the following composition:

Alcohol, 95 per cent	100.00 c.c.
Sodium nitroprusside	0.05 gm.
Benzidine, C. P. (Harmer)	0.05 gm.
Basic fuchsin	0.05 gm.
Hydrogen peroxide	0.5 c.c.

I have found this stain to be very satisfactory except for the fact that it is not stable. The hydrogen peroxide deteriorates very rapidly, and a

*From the Department of Clinical Pathology, University of Colorado School of Medicine.

staining solution which gives good results when fresh cannot be depended upon a few days later.

It was learned by experience that when this stain ceased to bring out the granules, the addition of the proper amount of hydrogen peroxide would restore the solution to its former potency. Subsequent additions, however, were valueless.

Because of these defects a modification of Goodpasture's stain was devised which gives good results for about eight months. After this period the granules in the positively reacting cells may still be seen, but they are not nearly as distinct as they should be.

The formula which I have found to give satisfaction is as follows:

Alcohol, 95 per cent	100.00 c.c.
Sodium nitroprusside	0.05 gm.
Benzidine, C. P.	0.05 gm.
Basic fuchsin	0.10 gm.

It will be seen that in this formula hydrogen peroxide is omitted, and the amount of the fuchsin is doubled. I have found, as mentioned by Todd,[2] that a clearer picture may be obtained by increasing the amount of fuchsin. This probably is due to a difference in the strength of the dye.

When it is desired to stain the blood smear, a 1:200 dilution of hydrogen peroxide is made in distilled water. This gives the same concentration as in Goodpasture's stain. Without previous fixation a noted quantity of the staining fluid is applied to the film. After one minute the same number of drops of the aqueous hydrogen peroxide solution is added and allowed to act from three to six minutes, depending upon the stain and upon the depth of staining desired. The preparation is then rinsed, dried, and examined. By this method the granules stand out very clearly in the cells which give the peroxidase reaction.

The hydrogen peroxide dilution keeps well for some weeks; but because such a small amount is needed, it is probably just as well to make it up fresh each time since the dilution is easily made by counting the drops from a medicine dropper.

REFERENCES

[1]Goodpasture, E. W.: A Peroxidase Reaction with Sodium Nitroprusside and Benzidine in Blood Smears and Tissues, Jour. Lab. and Clin. Med., April, 1919, iv, 442.
[2]Todd, J. C.: Clinical Diagnosis by Laboratory Methods, ed. 5, Philadelphia, 1923, W. B. Saunders Company, p. 323.

A MODIFICATION OF THE THALHIMER APPARATUS FOR THE SLOW INTRAVENOUS INJECTION OF GLUCOSE AND SALINE SOLUTIONS*

By George T. Pack, B.Sc., M.D., University, Ala.

IN THE treatment of severe postoperative acidosis or severe vomiting of pregnancy, Thalhimer has developed a method of treatment that is quite efficacious. It depends upon the slow intravenous injection of 1000 c.c. of a sterile 10 per cent glucose solution, followed by insulin hypodermically.[1] The time consumed in giving this amount is from four to five hours; the rate

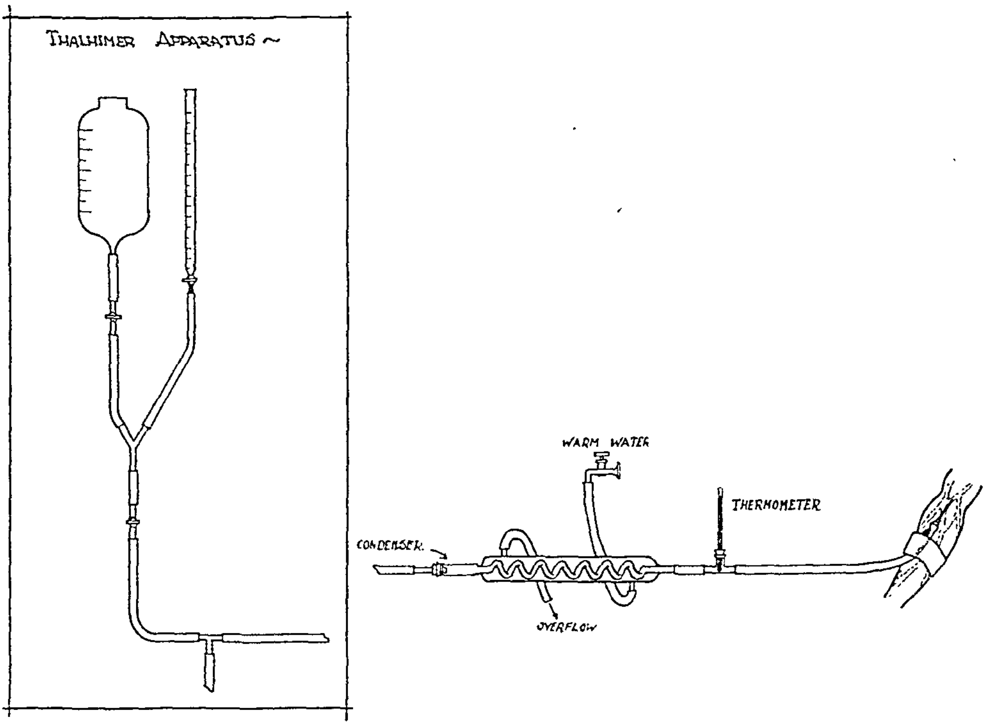

Fig. 1.

of introduction is controlled by reading the changing fluid levels in a graduated burette, fed at intervals from a main reservoir.[2]

This rate of flow is so slow that the solution must inevitably cool before it enters the vein. To enclose the reservoir with suspended hot water bags will not greatly obviate this defect. The temperature of the solution injected

*From the School of Medicine, The University of Alabama.
Received for publication, May 5, 1926.

should be at least 105 degrees F. (41 degrees C.). Even when the rate of flow is as rapid as 500 c.c. in ten minutes the temperature of the fluid will be lowered two degrees as it flows from the container to the vein of the patient. If the fluid is administered at the temperature of 115 degrees F. (46 degrees C.), the heat will exert a stimulating effect on the circulation and the sugar will be more readily utilized.

A method is illustrated for the maintenance of a high and equable temperature of the fluid. An ordinary glass condenser (sterile) is interposed in the circuit together with a thermometric attachment. The outer chamber of the spiral condenser is connected with slowly running hot water, the source of which is either a convenient hot water tap or a funnel to which hot water is added as needed. The thermometer is sterilized by immersion in a 1 to 500 mercury bichloride solution and inserted in the upright limb of a glass T-tube.

REFERENCES

[1]Thalhimer, W.: Surg., Gynec. and Obst., 1924, xxxix, 237.
[2]Thalhimer, W.: Jour. Am. Med. Assn., 1922, lxxviii, 190.
[3]Dutton, W. F.: Intravenous Therapy, Philadelphia, 1924, F. A. Davis, Publisher.

AN IMPROVED COLONY COUNTING APPARATUS*

By T. C. Buck and Jos. C. Swenarton, Baltimore, Md.

IN ORDER that one may be sure that he is counting the colonies on Petri plates under uniform conditions, it is essential that the illumination be standardized and, therefore, artificial.

The only colony counting apparatus we know of which is designed for use with artificial light is the one described by Stewart in the *Journal of Medical Research*, January, 1906. This apparatus, while very useful for some purposes, has certain drawbacks for use in the routine counting of many plates.

The chief objection to the apparatus for this work is that too much light is transmitted through the plate, thereby making transparent colonies difficult to see. It is also unsatisfactory to have the ruled guide plate above the colonies, because, on crowded plates, it is impossible accurately to keep track of which colonies have been counted and which have not been counted.

It was to overcome these objections that we undertook the construction of the apparatus described here. The design, which is shown in the accompanying cut, was evolved in a purely empirical way. That is, from our experience with the Stewart counting apparatus and the ordinary ruled background apparatus we were able to get ideas as to how the apparatus

*From the Bureau of Bacteriology, Health Department, Baltimore, Md.
Received for publication, February 20, 1926.

might be improved. Thus, after trying various combinations, we finally produced the design illustrated.

While our method of procedure was empirical, we believe that the principle involved is one of the correct combination of transmitted and reflected light. In this apparatus we get enough transmitted light to illuminate the agar, while the colonies are seen by reflected light. In the Stewart counting apparatus not only the agar becomes transparent but the colonies also. The result of the combination developed in this apparatus is that all colonies can be distinguished easily without eye strain.

DESCRIPTION

The apparatus consists of a box 9 × 11 × 2⅜ inches. This is fitted with a blue background plate. On top of the blue background plate is placed a

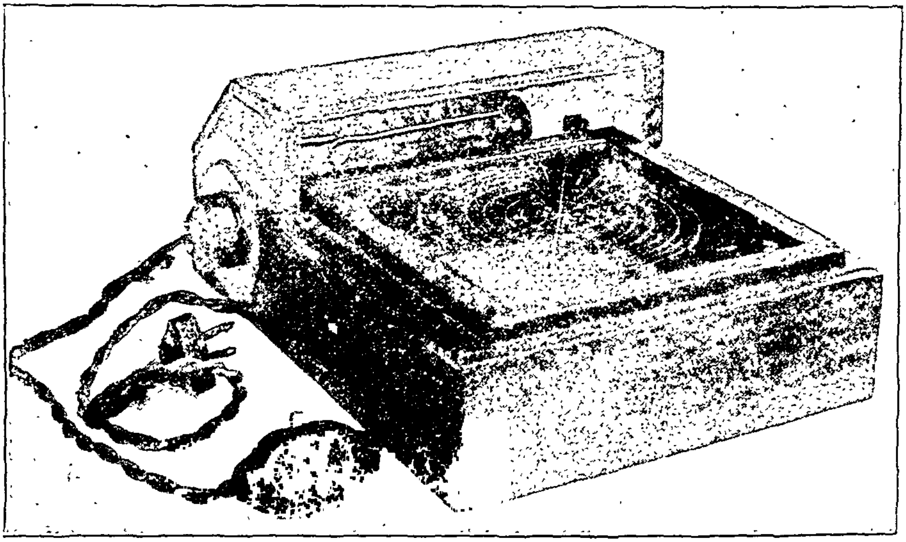

Fig. 1.

ruled Jeffer plate, which in turn is covered with a piece of plate glass. An electric light bulb furnishes the illumination. The inside of the box is painted white, and in the bottom under the glass plates is a reflector plate of glazed porcelain. The electric light is a 25 watt elongated bulb and is so placed that the longitudinal axis of the bulb is in the same plane as the middle glass plate. The bulb is covered with a reflecting hood which has a surface built at the proper angle to reflect the light onto the colonies. This hood also protects the eyes from the light. In order to make the transmitted illumination uniform, the end of the box away from the light is sloped so that some of the light is reflected back onto the glazed porcelain reflector. This tends to illuminate this reflector uniformly.

In making colony counts, the Petri dish is placed on the plate glass which covers the Jeffer plate and the colonies viewed through the regulation hand lens.

SUMMARY

A colony counting apparatus is described and illustrated which is superior to any on the market for the reasons:

1. It provides for a uniform illumination.
2. The colonies are illuminated in such a manner that they can easily be distinguished without eye strain.
3. It is simple in construction and includes no unnecessary parts which might interfere with the rapidity with which it can be used.

A DENTAL CONTRIBUTION TO THE BASAL METABOLISM TEST*

By John Bell Williams, D.D.S., and Margaret Nolting, M.D.
Richmond, Va.

MANY patients, because of the absence of natural or artificial teeth, or because of the abnormality of the alveolar ridges, present a problem to the operator in securing on them a correct basal metabolism rate. Such patients are unable to take a proper grip on the mouthpiece of the metabolism apparatus and a leakage results.

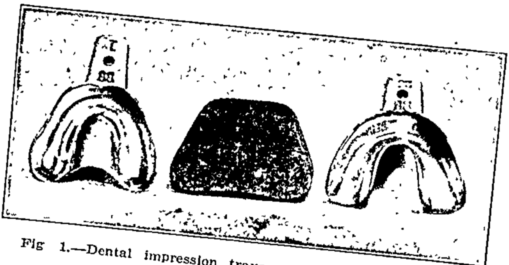

Fig 1.—Dental impression trays and impression material.

In our hands mouthpieces in general usage are not altogether satisfactory. With this in mind we sought a mouthpiece which would minimize leakage. Our appliance is based upon certain well-known dental principles. It is inexpensive and easily constructed, and with its aid patients can hold a mouthpiece with ease and comparative comfort.

The first step in constructing our appliance is shown in Fig. 1 which exhibits the typical dental impression trays, together with a hard impression material composed of stearin, gum dammar and powdered soapstone colored with carmine. This impression compound which is softened by mild heat is used for securing a correct impression of the alveolar ridges and hard palate. The impression is then removed from the mouth and tray (Fig. 2). The impression is returned, without the trays, to the mouth. Sufficient im-

*From the McGuire Clinic, Richmond, Va.

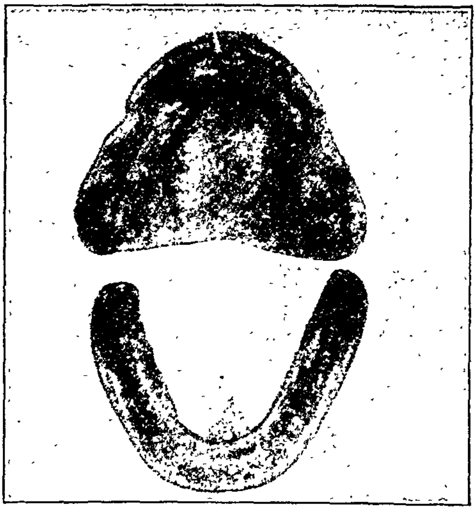

Fig. 2.—Impression material removed from the mouth and trays showing *imprint of alveolar ridges and hard palate.*

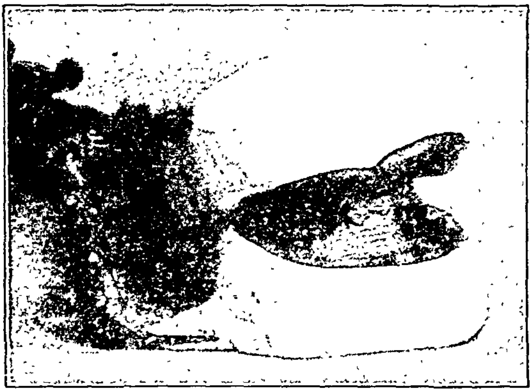

Fig. 3.—The finished appliance mounted on an articular.

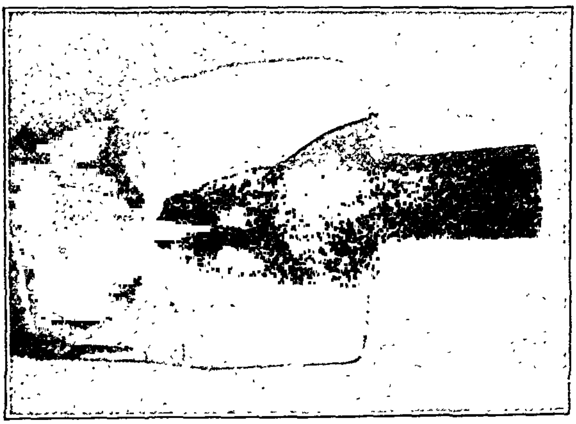

Fig. 4.—Showing the appliance and mouthpiece in proper relation.

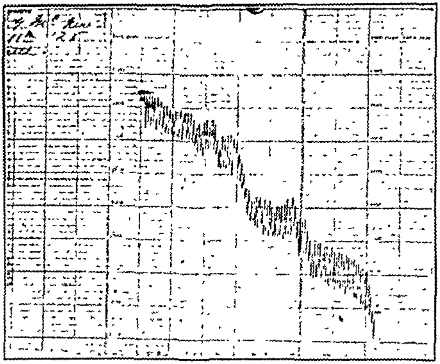

Fig. 5.—Shows an unsatisfactory tracing made with the mouthpiece retained by adhesive plaster.

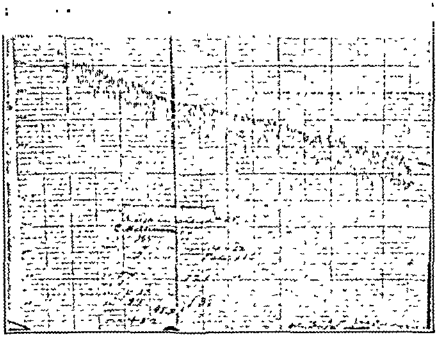

Fig. 6.—Shows a satisfactory tracing made with our appliance.

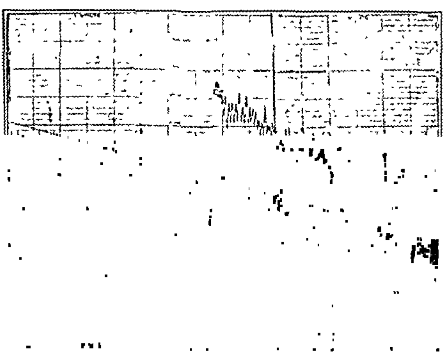

Fig. 7.—Shows an unsatisfactory tracing made without the appliance side by side with a satisfactory tracing made with the appliance.

pression material is added over the ridges in order to provide a breathing space in front and to seal them with the jaws in their proper relation. The impression is then mounted on an articulation. The ridges are serrated to grasp the mouthpiece (Fig. 3). The completed appliance with the mouthpiece in position is shown in Fig. 4. Fig. 5 shows a tracing with the mouthpiece retained in the usual way by means of adhesives. The tracing made for this same patient with the appliance in position is shown in Fig. 6. The value of this appliance is forcibly shown in Fig. 7, where one may compare side by side the tracings made without and with the aid of the appliance.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

Kusama, S.: On the Etiology of Typhus Fever and Measles. Japan Med. World, Nov. 15, 1925, v, No. 2, 309.

Kusama has been investigating this subject since an outbreak of typhus occurred in Tokyo in 1914.

Japanese monkeys which had contracted the disease by injection of the passage virus of typhus were bled to death and the kidneys removed aseptically.

A small portion of the kidney was placed in tubes containing 10 c.c. of ascitic broth and incubated at 37°C. for two to three weeks.

The medium consisted of one part of ascitic fluid to four parts of broth, the P_H being adjusted to 7.0.

In successful culture the tubes show turbidity in 10 to 14 days.

The organism thus cultivated is a bacillus 0.9 to 1.8 micra long and 0.4 to 0.5 micron in width. The ends may be sharp or dull, and the organism straight or slightly curved.

There may be perceptible pleomorphism.

Fresh cultures are stained with difficulty. In older, giessa-stained cultures the body of the organism is light blue and contains chromatic granules. The organism is gram-positive and nonacid-fast.

Subcultures are difficult at first but growth occurs later on various media, Loeffler's serum and potato.

Inoculation of this organism into monkeys produced a reaction somewhat similar, but less prolonged, to that following the injection of the passage virus of typhus. The histologic changes were also similar to those seen in experimental typhus in monkeys.

Cross immunity tests are recorded as positive by Kusama. Agglutinins, opsonins, or complement-fixing bodies were not produced by the organism.

After intraperitoneal inoculation the organism could be demonstrated for five days and then disappeared. Later, when the onset occurred, the organism could be recovered from the kidneys.

Using an identical technic, another organism was recovered from monkeys inoculated with blood from cases of measles.

It is a gram-positive bacillus, 1.2 to 2.3 micra long and 0.5 to 0.7 micron wide and morphologically very similar to the organism above described. It grows only on liquid media and is anaerobic; older cultures will grow aerobically on both liquid and solid media. It is chromogenic (pink).

It is apparently nonpathogenic and Kusama is attempting to show its relationship to measles by immunologic methods

Leuci, T.: The Hematoclastic Wassermann Reaction. Rev. Assn. Med. Argentina, October, 1925, xxxviii, 415.

Leuci investigated the theory advanced by Bancin in 1923 with reference to the reacting substances producing a positive Wassermann reaction, namely, that these were in part retained within the blood cells.

Bancin tested serum into which hemoglobin had been liberated by breaking up the clot.

Leuci collected blood in tubes containing glass beads which served to guard against coagulation, to defibrinate the blood, to mechanically rupture the corpuscles, and to obtain plasma instead of serum.

Leuci believes that the procedure increases the sensitivity of the complement-fixation reaction.

Drucker, P., and Allen, G. E.: A Simple Method for Obtaining Cutaneous (Capillary) Blood from Infants and Adults for Colorimetric P_H Determination. Jour. Biol. Chem., May, 1925, lxiv, No. 1, p. 221.

Details of Technic:

The knife, a cataract knife 2 x 30 mm. must be razor sharp. The incision is about 5 mm. deep and 2 mm. wide.

The tubes are clear glass 13 x 54 mm.

The indicator NaCl solution is prepared from 0.9 per cent NaCl from distilled water. It is desirable, but not necessary, to have this free from CO_2. About 45 c.c. are placed in a 50 c.c. measuring flask and *exactly* 0.54 c.c. of 0.06 per cent phenol red solution added.

One to three drops of 0.02 N NaOH are added to make the reaction P_H 7.4 to 7.6 and the solution diluted to 50 c.c. with NaCl solution.

(The saline solution must contain $\frac{54}{50}$ as much indicator as the standard, hence the 0.54 c.c. of indicator to allow for the 0.4 c.c. of blood.)

The standard solutions are prepared from Sorensen's phosphate at 0.05 P_H intervals, using 0.01 c.c. of the same indicator per 10 c.c. of standard solution.

Phenol-red stock solution: 0.1 gm. dissolved in 5.7 c.c. of 0.05 N NaOH added to about 80 c.c. of distilled water, boiled, diluted to 100 c.c. and filtered.

It is best to determine, for each lot of phenol red, what concentration gives the best difference in color.

Paraffin oil: Density 0.885.

The glass apparatus is best cleaned in sulphuric-bichromate solution.

Method:

Infants: Immerse the entire foot in hot water (45 to 50° C.), until a good hyperemia occurs.

Remove and dry with ether. Dip the heel into an ordinary glass funnel about 55 mm. in diameter, closed at the bottom of the V, and filled with paraffin oil.

Make a stab incision under the surface of the oil on the under side of the heel somewhat to one side of the midline.

Allow blood to collect under oil. With a 1 c.c. pipette graduated in 0.01 c.c. draw up 0.2 to 0.3 c.c. of oil and then 0.8 c.c. of blood.

Transfer the blood to a comparison tube containing 5 c.c. of 0.9 per cent NaCl, overlaid with oil, and another 0.4 c.c. to a tube of NaCl—phenol red solution, also overlaid with oil.

Stopper the tubes with a one-hole stopper so that all the oil is expressed, and close the hole with a glass plug.

Centrifuge 2 to 5 minutes and compare with the standards.

Adults: Specimen collected from ear or finger as described above.

Reading must be made within one-half hour.

Read the temperature of the diluted plasma.

The P_H at 38°C. is then calculated by the following formula:

$$P_H\ 38° = P_H \text{ color } T° \text{ plus } 0.01\ (t-20°) - 0.26$$

The 0.26 is Cullen's constant for human plasma plus the correction for loss of CO_2 in oil of 0.03 to 0.04 P_H. The 0.01 (t − 20°) is the correction for change in room temperature.

Bibb, L. B.: Long Tube Method of Cultivating Organisms, with Observations on Mobile Colonies in Liquid Medium. Jour. Bact., November, 1926, x, No. 6, p. 561.

The method devised by Bibb obviates mechanical and other disturbance incident to transfer and affords a continuous view of the reaction of bacteria to environment.

Ordinary glass tubing of 3 to 5 mm. outside diameter was autoclaved in 5 foot lengths which were sealed end to end in a blast lamp until a 30 foot tube was obtained.

Under aseptic precautions, and avoiding air bubbles, nutrient broth ($P_H 7.6$) was aspirated into the tube. One end of the tube was then sealed and the other cut off one inch longer than the column of broth.

One c.c. of the culture to be studied was then emulsified near the open end of the tube which was left in a horizontal position at room temperature. *B. coli* from 24-hour slants was thus studied.

The progress of the bacterial growth along the tube can be followed with the naked eye, a visible turbidity preceding by about ½ inch the most advanced bacilli.

The rate of progress was uniform and averaged about 4 feet per day or 5 cm. per hour.

The turbidity was opaque at the head of the column and when viewed in profile appeared as one or two triangular areas of dense, whitish turbidity separated by a streak of relatively clear medium, transverse to the tube, and about 5 mm. wide.

About 3 to 5 cm. in the rear of the head of the column appeared a second triangular turbidity, sometimes bifid or double. Between these two areas was a segment of clear medium 3 to 5 cm. long.

The characteristics of these turbid areas varied somewhat with different cultures but was the same for the same culture.

Bacilli of certain strains regularly formed mobile colonies of characteristic shape. If these were disturbed by agitation, they again formed into their usual shape in a few minutes.

The method furnishes a practical means for observing the effect of different substances or influences on bacterial growth and activity.

The paper is illustrated.

Pelouze, P. S., and Viteri, L. E.: A New Medium for Gonococcus Culture. Jour. Am. Med. Assn., March 6, 1926, lxxxvi, 684.

The authors maintain that much of the difficulty experienced in culturing the gonococcus and, perhaps, some of the cultural characteristics attributable to the organism, are due to lack of a suitable medium.

On the medium described below gonococcus culture is declared to be as easy as other ordinary routine cultures, the organism growing indefinitely on subcultures and retaining its vitality for at least one month.

The method of preparation follows:

A calf's brain, weighing approximately 500 gm., is forced through a wide-meshed gauze into 500 c.c. of distilled water and placed in the ice box for twenty-four hours. It is then filtered several times through cotton of varying degrees of compactness. The resultant fluid is turbid, no matter how often it is filtered. To this is added 0.5 per cent of acid sodium phosphate and 1 per cent of peptone. It can now be autoclaved at 15 pounds pressure for twenty minutes and kept as stock, or the final steps for its completion can be carried out.

To complete the medium it is necessary simply to add one part of the brain bouillon to three parts of standard 2.5 per cent agar medium made from veal broth, with the addition of 0.5 per cent of sodium chloride and 1 per cent of peptone. It should then be adjusted to a P_H of 7.8 which allows for a reduction of two points in autoclaving, 7.6 being the desired end-point.

The medium is then tubed, autoclaved and slanted. After it solidifies, the usual cotton plug is replaced by a sterile rubber cork to retain the water of condensation, and the medium keeps indefinitely.

When it is completed and cooled, some flocculation is present in the butt of the tube. This can easily be removed if the medium in bulk is placed in the autoclave, quickly brought to 15 pounds pressure, removed, filtered, tubed and replaced in the autoclave for the completion of sterilization. While this improves its appearance, it seems to make some change in it which causes more scanty growths; consequently, the authors have not made use of it, preferring a good medium to a pretty one.

Tozerski, E.: A Favorable Synthetic Medium for the Development of the Bacteriophage Element. Compt. rend. Soc. Biol., Nov. 27, 1925, xcii, 1285.

The following formula was devised for the purpose indicated:

Double distilled water	1000	c.c.
Succinate of ammonia	6	gm.
Saccharose	40	gm.
Potassium sulphate	1	gm.
Magnesium sulphate	1	gm.
Disodium phosphate	0.5	gm.

The growth of bacteriophage on this medium was very satisfactory.

Tanner, F. W., Devereux, E. D., and Higgins, F. M.: The Multiplication of Yeast and Yeastlike Fungi in Synthetic Solutions. Jour. Bacteriol., January, 1926, xi, No. 1, p. 45.

Fulmer and Nelson's medium F was found satisfactory for the purpose.
The formula follows:

Ammonium chloride	0.188	gm.
Calcium chloride	0.100	gm.
Sucrose	10.0	gm.
Dibasic potassium phosphate	0.10	gm.
Dextrin	0.60	gm.
Distilled water	100.0	c.c.

The ammonium chloride content is adjusted for incubation at 38° C. The flocculent precipitate of calcium phosphate (?) serves, through its disappearance, as an evidence of growth.
Growth occurs in this medium in the absence of "bios."

Damon, S. R.: A Note on the Spirochetae of Termites. Jour. Bacteriol., January, 1926, xi, 36.

The following technic was used for the demonstration of spirochetae:

1. Emulsify the gut contents of the termite in a drop of 0.4 per cent saline solution and expose to the fumes of osmic acid for 30 seconds.
2. Dry in air or by very gentle heating.
3. Flood the slide with a 1:5 solution of 50 per cent carbolfuchsin and 50 per cent anilin gentian violet. Stain 60 seconds.
4. Wash in water, dry, and examine.

The background is light pink and the organisms much darker as they take the gentian violet.

The forms seen are described and illustrated in six microphotographs.

Elman, R., and McMaster, P. D.: Studies on Urobilin Physiology and Pathology. IV. Urobilin and the Damaged Liver. Jour. Exper. Med., July, 1925, xliii, No. 1, p. 99.

A continuation of studies on dogs upon this subject, this paper being concerned with the sequelae of direct injury to the hepatic cells or obstruction to the ducts.

Previous studies have shown that in the dog urobilin is formed *only in the intestinal tract.*

When a total bile loss was produced through hepatic injury (chloroform or biliary obstruction) and intubation of the common duct, bile and feces remained urobilin-free for as long as four and one-half months, the total period of observation in ten animals.

In eight animals in which liver injury was induced by chloroform, phosphorous, or toluylene-diamine, but only a fraction of the bile was lost by intubation of a small hepatic duct, most of the bile thus flowing to the intestine, urobilin was a regular constituent of the

bile and stool. When the bile constituents are prevented from reaching the intestine but accumulate within the body, as occurs during total biliary obstruction, there is not only a general disturbance of the organism but a progressive injury to the liver leading to secondary cirrhosis.

Urobilinuria, however, fails to occur even though there is a marked bilirubinurea.

With local and partial biliary obstruction a moderate urobilinuria occurs but no bilirubinuria, due to the failure of the unobstructed hepatic tissue to remove urobilin from the blood as completely as bilirubin.

Elman, R., and McMaster, P. D.: Studies on Urobilin Physiology and Pathology. V. The Relation Between Urobilin and Conditions Involving Increased Red Cell Destruction. Jour. Exper. Med., November, 1925, xliii, No. 5, p. 619.

An experimental study conducted with dogs.

Much of the interest in urobilin arises from its appearance in the urine in diseases characterized by excessive destruction of red blood cells.

In accordance with the results of studies previously reported, urobilinuria does not occur when the entrance of bile into the intestinal canal is totally prevented, even when extensive blood destruction occurs.

When blood destruction occurs under conditions of partial bile loss, urobilinuria occurs.

Urobilinuria occurs during the extravasation of blood after operation but not during the absorption of extravasated blood from hematomas.

Excessive blood destruction during infections is associated with urobilinuria but its formation in the intestine is the essential factor.

Kirby-Smith, J. L., Dave, W. E., and White, G. F.: Creeping Eruption. Arch. Dermat. and Syph., February, 1926, xiii, No. 2, p. 137.

In a profusely illustrated article the authors describe the clinical symptomatology, appearance and treatment of a skin affection seen in Florida.

They have demonstrated by means of serial sections of tissues removed at biopsy that the disease is due to the entrance into the skin of a migratory nematode larva approximately 1/50 inch in length and 1/12,000 inch broad.

No definite conclusion can as yet be drawn regarding the host of the adult worm, but it is suggested that the rat may be the normal host and that poultry, cats, dogs, and other animals may be concerned.

MacCarty, W. C.: The Cancer Cell and Nature's Defensive Mechanism. Surg., Gynec. and Obst., December, 1925, 783.

MacCarty presents the observations leading to his belief that the cancer cell has distinct morphologic characteristics whereby it may be recognized.

These cells are described usually as they have been seen when dead for some time and after they have been subjected to the action of various reagents which produce cytologic changes.

MacCarty's observations were made upon living cells and upon fixed but not embedded cells by methods he has already described.

Cancer cells cannot always be distinguished from regenerative cells but this can be done frequently on the difference between the volume-relationship between nucleus and nucleolus and the whole cell, this being greater in the malignant cell.

The regenerative cell is more delicately constructed and its nuclear granules are finer.

Familiarity with the picture is essential to certainty of recognition. Cellular differentiation is an important factor in prognosis, but it is not the only factor.

The cancer cell in the living state is seen as an ovoid or spheroid body with no irregularity of "cell wall," nucleus, or nucleolus; the demarcations of the component parts of the cell are sharp and distinct; the granules are fairly uniform in size, and the mitotic figures are sometimes multipolar but not asymmetrical.

The article is clearly illustrated with line drawings and microphotographs, and the author's conception of the cancer cell is best understood by reference to and study of the illustrations.

Heidelberger, M., and Avery, O. T.: The Soluble Specific Substance of Pneumococcus. Jour. Exper. Med., July, 1923, xxxviii, No. 1, p. 73.

Heidelberger, M., and Avery, O. T.: The Soluble Specific Substance of Pneumococcus. Second Paper. Jour. Exper. Med., September, 1924, xl, No. 3, p. 301.

Avery, O. T., and Heidelberger, M.: Immunologic Relationships of Cell Constituents of Pneumococcus. Jour. Exper. Med., July, 1923, xxxviii, No. 1, p. 81. **Second Paper:** Jour. Exper. Med., September, 1925, xlii, No. 3, p. 367.

Heidelberger, M., Goebel, W. F., and Avery, O. T.: The Soluble Specific Substance of Pneumococcus, Third Paper. November, 1925, xlii, No. 5, p. 727.

Avery, O. T., and Morgan, H. J.: Immunological Reactions of the Isolated Carbohydrate and Protein of Pneumococcus. Jour. Exper. Med., September, 1925, xlii, No. 3, p. 347.

Avery, O. T., and Neill, J. M.: The Antigenic Properties of Solutions of Pneumococcus. Jour. Exper. Med., September, 1925, xlii, No. 3, p. 355.

Heidelberger, M., Goebel, W. F., and Avery, O. T.: The Soluble Specific Substance of a Strain of Friedlander's Bacillus. Jour. Exper. Med., November, 1925, xlii, No. 5, p. 701.

Part II: Chemical and Immunological Relationships of Pneumococcus Type II and a Strain of Friedlander's Bacillus. Jour. Exper. Med., November, 1925, xlii, No. 5, p. 701.

The papers, the titles of which appear above, form a record of observations during the past several years, the salient features of which may thus be summarized.

In 1917 Dochez and Avery showed that whenever pneumococci were grown in fluid media, there was present in the culture fluid a substance which was precipitated by homologous antipneumococcus serum. A similar or analogous substance was demonstrated in the blood serum, urine, and body fluids of animals and human beings infected with pneumococci.

It was found possible to isolate this substance by precipitation with alcohol, acetone, and ammonium sulphate and to demonstrate that it consisted of a carbohydrate which appeared to be a polysaccharide built up of glucose molecules.

In the course of the isolation of this specific carbohydrate substance an additional substance, precipitable in the cold by acetic acid and consisting of nucleoprotein and mucoid was encountered.

This protein fraction of the bacterial cell was not type specific but reacted with antipneumococcus serum regardless of type derivation, thus differing from the carbohydrate fraction.

Further studies developed a method of securing the carbohydrate substance in a more purified form. The details of this newer method are somewhat lengthy and must be found in the original paper (Jour. Exper. Med., September, 1924, xl, No. 3, p. 301).

This purified product contains less than 0.2 per cent of nitrogen and is identified as a polysaccharide.

Immunologic studies of these two components of the pneumococcus cell (Jour. Exper. Med., September, 1925, p. 367) have shown that the carbohydrate substance is the type-specific substance, being chemically distinct and serologically specific for each of the three fixed types. The protein fraction, on the other hand, is common to all the pneumococci and is species-specific but not type-specific.

The carbohydrate fraction is nonantigenic; injection of the protein fraction produces an immune serum reacting with pneumococcus protein in general. These antiprotein sera do

not agglutinate type-specific strains and do not precipitate solutions of the carbohydrate substance.

Morphologic integrity of the bacterial cell is hence necessary for the expression of its full antigenic power.

The elaboration of the specific carbohydrate substance is a specialized function related to the capsular development and is most marked under optimal growth conditions.

In a continuation of these studies the type-specificity of the carbohydrate substance was demonstrated, that from each of the three fixed types differing from the others chemically, but all possessing a definite immunologic relationship.

Intact pneumococci give rise to agglutinins of homologous type and to precipitins for their type-specific carbohydrate.

Solutions of pneumococci fail to stimulate the formation of type-specific antibodies due to changes occurring during the dissolution of the cell.

The antigenic properties of solutions of the isolated protein and carbohydrate substances have been noted above.

It is suggested that if final proof can be brought for the conception that the capsular zone of the organism is largely composed of a carbohydrate specific substance, is part of the defense mechanism of the cell, and the site of its initial contact with antibody, then these soluble polysaccharides acquire a new significance, not only in the serologic reactions of the cell, but in the actual processes of infection and immunity in the host.

Further studies (Jour. Exper. Med., November, 1925) demonstrated that the polysaccharide material isolated by Toenissen in 1901 from the capsular material of B. Friedlander was closely related to the specific carbohydrate secured from the pneumococcus and the principle sugar from which the specific substance of Friedlander's bacillus was built up.

This substance and a similar substance from Type III pneumococci were found to be reciprocally precipitable in each other's antiserum.

The strain of Friedlander's bacillus and the Type III pneumococcus were found to be agglutinable in each other's antiserum, this relationship, however, not extending to all members of the Friedlander group.

Agglutinin absorption was not reciprocal, only those for the absorbing organism being removed. While cross precipitin reactions occur the absorption of precipitins was not reciprocal.

Protection experiments in mice were reciprocal and heterogenetic specificity is thus evidenced.

It appears probable that when the analogous specific polysaccharides of unrelated microorganisms correspond in chemical constitution, an immunologic correspondence results and a new avenue for the study of bacteria as disease-producing agents is thus opened up.

The methods for the isolation of the specific substances discussed are given in detail but are too lengthy to be abstracted satisfactorily.

Lutz, A.: A Method for Transferring Tubercle Bacilli from Solid to Liquid Culture Media. Am. Rev. of Tuberc., November, 1925, xii, No. 3, p. 270.

Fifty c.c. of glycerin agar is placed in a 100 c.c. Erlenmeyer flask. After sterilization the medium is allowed to harden in a slant running from just below the mouth to the bottom of the slant. When the slant is hardened, 15 c.c. of sterile glycerin broth is added; the flask is then sealed with a paraffined stopper and stored in an upright position.

A heavy inoculation is made on the slant down to the fluid level. A better growth is obtained if the plug is not sealed in but placed in lightly for the first two days of incubation. The growth covers the solid surface and spreads out over the surface of the broth by continuity.

If the spread is poor a portion of the pellicle may be pushed off onto the broth. Once there is growth on the broth, transfer to other broth cultures is easy.

BOOK REVIEWS

(Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.)

*The Biology of the Protozoa**

THE underlying principle in this presentation—the fruit of many years of research and teaching—is the irritability of protoplasm, combined with protoplasmic organization, the latter being specific in type.

This fundamental conception as applied to the protozoa is fully developed by the author for the purpose of developing a common point of view from which new observations and experiments may be evaluated.

While somewhat controversial at times, the monograph presents an analysis of a wealth of careful studies and observations, both those of the author and of protozoologists in general, and should be not only of interest but of great value to advanced students of this subject.

Insulin, Its Use in the Treatment of Diabetes†

THIS very excellent contribution is not only a critical summary of the insulin work done up to the present time and a presentation of our knowledge of its physiology and pharmacology but also and particularly it is a monograph on the treatment of diabetes, both with diet and with insulin. The first section, written by Dr. MacLeod, carries an historical résumé of the development of insulin and the presentation of our present knowledge of its action. From it we learn that there is much yet to be known of its mode of action. *Insulin appears to accelerate the rate at which sugar passes into* the tissues, but exactly what happens to the sugar after it has reached the tissues is uncertain. Apparently some compound is made with phosphorus. Evidently there is an interdependence between sugar and phosphorus in the body. In this connection it is of interest to note the work of Embeden, showing that a compound of phosphoric acid with a complex yielding lactic acid, undergoes decomposition as a result of muscular exercise.

The work of MacLeod and his associates on depancreatized dogs, which eventually die with symptoms of acute liver breakdown even though maintained on adequate insulin treatment but which are kept alive and apparently well for an indefinite period when the meat eaten is changed to raw pancreas, is most interesting. The small amount of active trypsin that ap-

*The Biology of the Protozoa. By G. N. Calkins, Ph.D., Professor of Protozoology, Columbia University. Pp. 623. 238 engravings. Cloth. Price $7.50. Lea and Febiger, Philadelphia.

†Medicine Monographs. Vol. VI. Insulin, Its Use in the Treatment of Diabetes. Part I. Physiology. By Dr. J. J. R. MacLeod. Part II. Clinical Section. By W. R. Campbell, M.D. Pp. 242. Cloth. Williams & Wilkins Co., Baltimore, 1925.

parently gets into the system following this method, evidently prevents the untoward end-results.

MacLeod reaches no clear-cut conclusions with regard to the action of insulin. By the time the second edition of the book goes to press he will have much to add. On the other hand, we venture to predict that little of what has been written will require changing.

The second and by far the larger portion of the book is devoted to the clinical section and is written by Dr. W. R. Campbell. It is the best exposition of the clinical action and use of insulin which we have as yet met. The procedures for regulating the diet and the diet tables are distinctly original and greatly simplified. They are such that even the physician who treats but an occasional diabetic can ill afford to dispense with the assistance which he will derive from a study of this contribution.

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*Lead Poisoning**

LEAD poisoning, now a relatively infrequent condition, but one which in the past has been of greatest importance, yet another of those diseases "first described by Hippocrates," is a clearly defined clinical entity, as a rule easily recognized. And yet our knowledge of the modus operandi of this inorganic poisoning is still quite obscure.

In years past lead poisoning occurred quite generally following the use of pewter dishes, the drinking of water which had passed through lead pipes, and the taking of lead medicinally. Even yet industrial lead poisoning is a factor to be taken into account. One of the great achievements of industrial medicine has been the elimination of much of this type of illness, but scarcely more than a year ago there was a veritable epidemic of industrial poisoning from tetraethyl lead.

Aub and his collaborators have studied the problem of lead poisoning from all angles and have collected their results into a comprehensive monograph. The chemical studies are particularly interesting. They find that lead exists in the blood in only a very small amount. Even in severe lead poisoning 2.8 mg. of lead per liter of blood is a high reading. The usual conception is that lead exists as an albuminate, but they conclude that in the blood it is present in colloidal suspension rather than in solution and in an inorganic form, namely, colloidal lead phosphate. They find that the only storage depot of importance is the bones, where lead is deposited again as the phosphate, replacing calcium.

Probably the most common source of absorption is through the intestinal tract, but absorption also definitely occurs through the respiratory tract, and when it occurs in this way, it produces more rapid and serious symptoms. In part this is probably due to the fact that the introduction is directly into the systemic circulation, while with gastrointestinal absorption the lead passes

*Medicine Monographs. Vol. VII. Lead Poisoning. By Joseph C. Aub, Lawrence T. Fairhall, A. S. Minot and Paul Reznikoff, with a chapter on the prevalence of industrial lead poisoning in the United States by Alice Hamilton. Pp. 265. Cloth. Williams & Wilkins, Baltimore, 1926.

through the liver where a good part of it is removed and excreted in the bile. The authors do not agree that lead cannot be absorbed through the skin but leave this open as a moot question. It is excreted through various channels, the chief of which is the gastrointestinal tract.

The authors find that the excretion of lead is controlled by essentially the same factors which govern calcium excretion. Magnesium, sodium, and potassium salts exert a decalcifying effect, dependent upon the fact that the organism tends to maintain a normal ratio between the concentration of these various salts in the body fluids. Better results in the removal of both calcium and lead, however, were obtained with acids, particularly those which cannot readily be burned in the organism but must be neutralized by the body bases. These are such as hydrochloric acid, either as such or as acid phosphate, and ammonium chloride, which on absorption produces hydrochloric acid. Alkali, on the other hand, tends to conserve calcium and lead, but if sufficient is given to produce an alkalosis, the tertiary lead phosphate is rendered more soluble, and so, here too, the excretion of lead is promoted.

Experimentally, even after the excretion of lead has been promoted for a long time, chemical examination of the bones shows considerable residual storage of the element. In its insoluble form in bone it apparently causes no trouble, and the authors raise the question as to whether it might not be better to favor complete storage of lead in the bones by high calcium intake and a careful maintenance of normal acid-base equilibrium.

Potassium iodide likewise favors excretion of lead, apparently chiefly because of the action of the iodine ion, but it is not as efficacious as the acids and acid-forming salts. The latter are more effective when the calcium intake is low.

The stippling of red blood cells, anemia, increased resistance to hypotonic saline and kindred phenomena appear to be dependent upon surface contact of the lead salt with the red cells. The so-called lead palsy is not a true neuritis but is dependent for its origin upon muscular damage and occurs more frequently in those muscles which are used more generally. Lead inhibits the motility of smooth muscle while it increases its tone.

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EDITORIALS

Dye Therapy

IN RECENT years a great deal of attention has been paid to the possible treatment of infection by the intravenous use of certain dyes, and some difficulty has been experienced in explaining satisfactorily the somewhat conflicting reports which have accumulated.

In any consideration of the subject it is necessary to distinguish between those dyes which act upon bacteria as dyes and whose effects are due to various radicals in their formula, and those whose action is due to bactericidal elements combined with or forming the dye

Representative of the former group is gentian violet, a member of the triphenylmethane group of dyes, whose bacteriostatic and bactericidal activities are restricted to certain groups of bacteria and even, at times, to certain strains of a single group. Illustrative of the latter group is mercurochrome, a combination of a metal and a fluorescent dye, whose bactericidal effects are due to the mercury in the combination.

In view of these inherent and important differences, it is of advantage to consider them separately.

The use of gentian violet arose from the work of Churchman who demonstrated by the study of pure bacterial cultures that, when exposed to the action of this dye, four phases of bacterial inhibition might be observed: namely, (1) cessation of motility; (2) inhibition of reproduction; (3) suspension of bacterial activity for varying periods; and (4) inhibition of sporulation.

Of very great interest is the fact that, by varying the proportions of the dye, any or all of these forms of inhibition may be produced, and of still greater interest the further fact that, once released from the influence of the dye, the bacteria may resume all their normal and usual activities.

To express this power to inhibit bacterial activity the term *bacteriostosis* has been coined.

Churchman's studies have shown that gentian violet possesses bacteriostatic properties, that these are most marked for gram-positive organisms, and that the dye may even be bactericidal.

He has also shown that acid fuchsin has an action contrary to that of gentian violet, and that, by a mixture of dyes, the combined and complementary action of both may be obtained.[1]

A satisfactory explanation of the mechanism of these effects of gentian violet upon bacterial cultures remains to be found. Apparently, it is not so much a question of the penetration of the dye into the bacterial cell and possibly more closely related changes—perhaps a change in the P_H—effected by the dye at the surface of the organism.[2] In the case of acid fuchsin, the bacteriostatic effects have been ascribed tentatively to the presence of SO_3 radical in its formula.[3]

The therapeutic application of these studies was inevitable and very definite, and excellent results were obtained in the treatment of local infections (diphtheria carriers, etc.), and in the treatment by injection of infections in closed cavities, such as empyema and joint cavities.

From this to the treatment of conditions characterized by or associated with bacteriemia was but a step, and it is in this field that disconcerting discrepancies between *in vitro* and *in vivo* results have been encountered, the consideration of which is attracting deserved attention.

Chemotherapy has always been influenced by and associated with the ideas of Ehrlich and until recent times was dominated by two conceptions: of a therapia sterilans magna and a specific chemical affinity between the drug and the organism. These conceptions, however, have been modified necessarily by the observations of more recent times.

It is not to be denied that very definite, and at times even dramatically startling, results have been obtained after the intravenous administration of gentian violet and mercurochrome. On the other hand, it cannot be gainsaid that equally striking and contradictory failures have been reported.

Some of these contradictions may be explainable by lack of controlled

observations, unsupported assumptions, and, as Churchman has pointed out,[4] by a lack of definition as to exactly what constitutes a septicemia.

While the term includes and indicates a bacteriemia, differentiation must be made between transient bacteriemias, such as may occur in health, and bacteriemias associated with such diseases as pneumonia or typhoid fever, which are clinical and pathologic entities, and those conditions which, beginning as localized or focal infections, terminate in or are complicated by extensive, repeated, continuous, or massive bacterial invasion of the blood stream.

As indicated by the above terms, it is clear that *septicemia* may include a variety of conditions, the gravity of which is influenced by a variety of factors. Among these may be mentioned: (a) the virulence of the invading organism; (b) the accessibility of their source of origin—the focus of infection—to treatment; (c) the resistance of the patient; and (d) the site and gravity of the secondary lesions resulting from bacterial localization in various organs or tissues of the body.

It is fallacious, as Churchman comments, to assume that, in septicemia, bacteria travel round and round in the blood stream reproducing as they go, and that injected antibacterial substances travel with them. The bacteria originate in and enter the blood from some focus; they are removed from the blood by the defensive mechanism, which includes the mechanical filtration by lymph glands, etc., as well as specific antibodies, phagocytes, and the like.

It is obvious that there are many factors entering into and influencing the end-results of septicemia and influencing both the successes and failures which have been reported.

It can be stated that the beneficial effects reported are not to be explained upon the basis of a therapia sterilans magna nor as resulting entirely from a specific bactericidal effect of the dye upon the organism while both are coursing through the blood stream.

Churchman and Herz,[5] for example, have shown that gentian violet, even when injected in massive doses, rapidly disappears from the blood, and Churchman has recorded his inability to kill organisms in the blood by the injection of gentian violet.

Brill and Myers[6] found the maximal advisable concentration of gentian violet or mercurochrome in the blood stream—1.10,000—without bactericidal action upon staphylococci, streptococci, or the colon bacillus.

Meleney and Zan[7] were unable to demonstrate any bactericidal action of neutral acriflavine on hemolytic streptococci, even when injected in lethal doses in rabbits. Walker,[8] in an investigation of the bactericidal properties of freshly defibrinated blood to which mercurochrome was added in varying concentrations, found no increase in bactericidal activity toward the colon bacillus in concentration of mercurochrome as high as 1:400; with concentrations of 1:200 the bactericidal activity of the blood was destroyed.

Staphylococci and streptococci were found to grow more luxuriantly in the mercurochrome-blood mixture (1:400) than in plain blood, due, apparently, to the injurious action of mercurochrome on leucocytes.

Other and similar reports are to be found in the literature to confront and confuse the observer likewise confronted with clinical reports of definite and striking "cures" and recoveries.

An important angle of the question, which has only recently begun to receive the attention it deserves, is the fact that chemotherapy, as Kolmer has said, may be associated with chemopathology.

Young, Scott and Hill[9] have reported that 900 mg. of mercurochrome may be administered daily by mouth for ten days without injury, and Hill and Bidgood[10] state that definite renal damage does not follow intravenous administration of mercurochrome unless the dose is at least 10 mg. per kg.; the slight damage done by smaller amounts is not irreparable.

St. George,[11] however, in necropses of five cases treated with mercurochrome, found in all intense nephritis and intense congestion of the cecum and superficial colonic ulcerations characteristic of mercurial poisoning, mercury being recovered from the viscera in amounts larger than those seen in bichloride poisoning. The largest amount of mercurochrome administered was 0.9 gm. (90 c.c. of 1 per cent solution) the smallest, 0.1 gm. (20 c.c. of 0.5 per cent solution).

Corper[12] has shown that the intracutaneous injection of 0.2 c.c. of 0.5 per cent mercurochrome produces ulceration; that intratracheal injection produces distinct pathologic changes persisting for as long as four days, the severity varying with the amount and concentration of the solution; and that intrapleural injection may produce permanent parenchymal damage.

It is apparent, therefore, that these agents not only cannot be expected to produce a therapia sterilans magna, but that they must be used with care and due regard for the cumulative and even dangerous chemopathology which may result.

The mechanism of the clinical results observed still remains to be explained.

In the case of gentian violet, Burke and Hewton[13] have shown that the intensity of the bacteriostatic and bactericidal activity of this dye may be influenced by variations in the character of the solutions; the more alkaline the solution, the greater the bactericidal activity, and vice versa. As a stable alkaline solution cannot be prepared, they suggest the use of a buffered solution near neutrality or a *freshly prepared* solution in 3 per cent sodium bicarbonate.

It is not known just how dyes produce cellular death nor what is the relation of alkalinity to this reaction. Burke and Grieve,[14] however, have shown that the bacteriostatic and bactericidal activity of many dyes varies with the P_H of the medium surrounding the organisms and that, within certain limits, alkalinization of the body fluids may augment the action of the dye.

In the face of the discordant data, the contradictory character of which has been indicated, Churchman[4] has summed up the possible explanations of the results on record as follows:

Admitting that the dyes can be introduced into the circulation only in relatively small amounts, that they are rapidly removed as such, and that it is hardly conceivable that they remain in contact with bacteria for a time sufficient to permit specific antibacterial action, there still remains the possibility that they may be changed in the body into substances more potent than the mother dye. Or, again, it is possible that they may stimulate the production of antibacterial substances in the tissues.

Still further, as both bacteria and dye stuff are removed from the blood stream by various agencies, it is possible that in various organs or foci a more prolonged and intimate contact of bacteria and dye may occur and so bactericidal or bacteriostatic reactions may be encouraged.

Of very great importance, perhaps, is the *bacteriostatic* activity of dyes as opposed to or contrasted with their bactericidal powers, by means of which the bacteria are inhibited, their growth and activities held in abeyance; opportunity is thus afforded for the various defensive mechanisms of the body to be mobilized to come into play.

A still further theory is that the activity of toxins may be affected or restrained by dyes, in the case of fluorescent compounds, such as mercurochrome, perhaps, by some photodynamic action. This, however, is entirely speculative and remains to be demonstrated.

All that can be said so far is that a therapia sterilans magna cannot be achieved by the use of dyes and; indeed, there are factors, such as the possible sudden liberation of massive doses of endotoxin, which render this not always desirable.

The bacteriostatic powers of both gentian violet and mercurochrome appear to be of more therapeutic importance than their bactericidal abilities, and the ultimate results consequent upon their use depend in no small measure upon the resisting powers of the patient to which, in all probability, they bring increased opportunity for mobilization and attack upon the invading bacteria, temporarily inhibited by the bacteriostatic effect of dye therapy.

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CLINICAL AND EXPERIMENTAL

ON THE PROCESS OF LYMPH FORMATION*

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WE HAVE met no satisfactory theory on the process of lymph formation, though some theories have been published, such as the filtration theory of Ludwig,[1] the secretion theory of Heidenhain,[2] and the cellular physiologic theory of Asher.[3]

Lymphagogues as defined by Heidenhain[2] are substances which, injected into the blood stream, cause an increased flow of lymph from the thoracic duct. He divided them into two classes. The first class includes such substances as peptone, leech extract, crayfish extract, etc. These bodies cause an increased flow of more concentrated lymph. The second class includes such substances as sugar, salt, urea, etc. These increase the lymph flow, the lymph becoming more watery than before.

It will be seen that there is an obvious difference between the lymph produced by either class of lymphagogues; therefore, it is better to study their actions on the basis of the two classifications.

THE SE D CLASS OF LYMPHAGOGUES

Heidenhain[2] found that so ns of sodium chloride, sodium nitrate, sodium sulphate, and sodium iodide of the sam rcentage are not equally effective in increasing lymph flow; sodium chloride with the lest molecular weight is the most effective, sodium nitrate less so, sodium sulphate still l and sodium iodide with the largest molecular weight the least effective. He conclude erefore, that the effects on increasing lymph flow are parallel to the water-attractin wer of the solutions.

Using equimolecular sol s, instead of those of the same percentage, Heinz[4] found that sodium chloride and so iodide are equally effective.

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I have studied solutions of different members of the second class to determine whether they are equally effective in increasing lymph flow, whether their water-attracting power was the same.

In aqueous solution, sodium chloride and sodium iodide dissociate into two ions, sodium sulphate into three ions, and sodium phosphate into four ions, while glucose does not dissociate at all. Therefore the solutions of these substances will be nearly of the same osmotic pressure if their concentration is as follows.

Sodium chloride	½ mol	2.9 per cent
Sodium iodide	½ mol	7.4 per cent
Sodium sulphate (crystal)	⅓ mol	10.7 per cent
Sodium phosphate (crystal)	¼ mol	8.9 per cent
Glucose (anhydrous)	0.92 mol	16.6 per cent

Injecting each of the above solutions, 5 c.c. per kg. of body weight, into the vein of dog, I observed the influence on the lymph flow and the urine output.

The lymph flowing out of the thoracic duct was put into graduated cylinders and measured every ten minutes. At the same time the urine was obtained by a catheter retained in the bladder.

To compare the effects of each solution, I have used the following proportion showing it by L.

$$L = \frac{\text{Lymph quantity in 30 minutes after the injection}}{\text{Lymph quantity in 30 minutes before the injection}}$$

TABLE I

Gray bitch, 5.5 kg. Fasted 36 hours before the operation. Narcotized with paraldeyde (6.6 c.c. in 55 c.c. of water).

TIME	LYMPH C.C.	URINE C.C.	NOTICE
1.30′–40′	2.3	0.8	
40′–50′	2.1	0.8	
50′–60′	2.0	0.8	
2. 0′–10′	2.0	0.8	
10′–20′	2.4	0.8	
20′–30′	1.7	0.8	
30′–40′	4.6	3.4	30′–31′15″
40′–50′	4.8	2.4	Sodium chloride (2.9 per cent)
50′–60′	4.6	0.8	27.5 c.c. intravenously
3. 0′–10′	4.3	0.8	
10′–20′	4.1	1.0	
20′–30′	2.8	1.0	
30′–40′	3.3	1.0	
40′–50′	2.6	1.0	
50′–60′	2.7	1.2	
4. 0′–10′	2.2	1.0	
10′–20′	1.7	0.8	
20′–30′	1.6	0.8	

$$L = \frac{4.6 + 4.8 + 4.6}{2.0 + 2.4 + 1.7} = 2.29$$

In Tables I and II we see that ½ mol solution of sodium chloride and ⅓ mol solution of sodium sulphate are nearly equally effective in increasing lymph flow but that sodium sulphate is much more effective than sodium chloride in increasing urine output.

TABLE II

Piebald bitch, 5.5 kg. Fasted 36 hours before the operation. Narcotized with paraldehyde (6.6 c.c. in 55 c.c. of water).

TIME	LYMPH c.c.	URINE c.c.	NOTICE
11.25′–35′	2.2	0.8	
35′–45′	2.3	0.8	
45′–55′	2.2	0.8	
55′–65′	2.2	1.0	
12. 5′–15′	2.3	1.0	
15′–25′	2.1	1.0	
25′–35′	4.9	22.4	25′0″–26′15″
35′–45′	5.8	10.6	Sodium sulphate (10.7 per cent) 27.5 c.c.
45′–55′	4.3	6.4	intravenously
55′–65′	3.1	4.6	
1. 5′–15′	3.0	4.4	
15′–25′	2.1	2.8	
25′–35′	2.1	2.6	

$$L = \frac{4.9 + 5.8 + 4.3}{2.2 + 2.3 + 2.1} = 2.27$$

TABLE III

Yellow bitch, 6.8 kg. Fasted 36 hours before the operation. Narcotized with paraldehyde (8.2 c.c. in 68 c.c. of water).

TIME	LYMPH c.c.	URINE c.c.	NOTICE
12.10′–20′	2.8	2.4	
20′–30′	2.8	2.0	
30′–40′	2.4	2.2	
40′–50′	2.2	2.6	
50′–60′	2.1	2.4	
1. 0′–10′	2.2	2.2	
10′–20′	4.7	14.6	10′0″–11′15″
20′–30′	3.7	6.8	*Sodium phosphate (8.9 per cent)*
30′–40′	3.5	6.0	34 c.c. intravenously
40′–50′	2.6	3.4	
50′–60′	2.2	2.2	
2. 0′–10′	1.9	2.4	
10′–20′	2.0	2.8	
20′–30′	1.9	2.2	

$$L = \frac{4.7 + 3.7 + 3.5}{2.2 + 2.1 + 2.2} = 1.83$$

TABLE IV

Black bitch, 7.8 kg. Fasted 36 hours before the operation. Narcotized with paraldehyde (8.4 c.c. in 78 c.c. of water).

TIME	LYMPH c.c.	URINE c.c.	NOTICE
12.10′–20′	3.2	0.8	
20′–30′	3.1	1.0	
30′–40′	3.2	0.8	
40′–50′	3.1	0.8	
50′–60′	3.2	1.0	
1. 0′–10′	3.1	0.9	
10′–20′	7.6	10.8	10′0″–11′30″
20′–30′	7.9	8.0	Glucose (16.6 per cent) 39 c.c.
30′–40′	4.8	3.2	intravenously
40′–50′	4.4	1.6	
50′–60′	3.3	1.2	
2. 0′–10′	2.9	1.2	
10′–20′	1.9	0.8	

$$L = \frac{7.6 + 7.9 + 4.8}{3.1 + 3.2 + 3.1} = 2.15$$

TABLE V

Yellow bitch, 8.6 kg. Fasted 36 hours before the operation. Narcotized with paraldehyde (10.3 c.c. in 86 c.c. of water).

TIME	LYMPH C.C.	URINE C.C.	NOTICE
2.45′–55′	3.5	3.2	
55′–65′	3.5	3.2	
3. 5′–15′	3.5	3.2	
15′–25′	3.5	3.2	
25′–35′	3.2	3.2	
35′–45′	3.0	3.2	
45′–55′	2.9	3.0	
55′–65′	6.2	10.0	55′0″–56′30″
4. 5′–15′	6.7	7.0	Sodium iodide (7.4 per cent) 43 c.c.
15′–25′	6.1	8.4	intravenously
25′–35′	4.4	7.4	
35′–45′	4.0	7.2	
45′–55′	4.0	7.2	
55′–65′	3.7	7.3	
5. 5′–15′	3.6	7.2	
15′–25′	3.9	6.5	
25′–35′	4.0	6.5	
35′–45′	3.4	6.3	
45′–55′	3.6	5.4	
55′–65′	3.3	4.9	

$$L = \frac{6.2 + 6.7 + 6.1}{3.2 + 3.0 + 2.9} = 2.08$$

The effects of glucose and sodium iodide are nearly the same as those of sodium chloride and sodium sulphate, but sodium phosphate is a little less effective than the others. This is probably due to the fact that sodium phosphate does not dissociate completely into 4 ions but rather partly into three, i.e., two Na′ and one HPo_4''. Therefore the ¼ mol solution of sodium phosphate is of less osmotic pressure than the other solutions.

In 11 cases the results were nearly the same, as we see in Table VI.

TABLE VI

SOLUTION	SODIUM CHLORIDE	SODIUM SULPHATE	SODIUM PHOSPHATE	GLUCOSE	SODIUM IODIDE
	2.29				
		2.27			
	1.65 ---------	1.75			
		2.15			
			1.83		
L			1.77		
	2.06 ---------	---------	1.87		
	2.01 ---------	---------	---------	1.89	
	1.97 ---------	---------	---------	2.15	
				1.97 ---------	2.18
					2.08
Average	1.99	2.06	1.82	2.00	2.13

(------) shows that two kinds of the solutions were injected one after the other in the same dog.

The effects on increasing lymph flow are nearly the same in all these cases. The increase of urine output is, however, not the same in all cases. Sodium sulphate causes the greatest increase, glucose and sodium phosphate next, and sodium iodide and sodium chloride least.

I examined further with more concentrated solutions.

TABLE VII

Black bitch, 12.7 kg. Fasted 36 hours before the operation. Narcotized with paraldehyde (15.2 c.c. in 127 c.c. of water).

TIME	LYMPH c.c.	URINE c.c.	NOTICE
5.20'–30'	4.9	3.2	
30'–40'	4.9	3.2	
40'–50'	4.8	2.8	
50'–60'	4.6	3.2	
6. 0'–10'	4.0	3.0	
10'–20'	4.0	3.0	
20'–30'	10.1	14.0	20'0"–21'45"
30'–40'	12.3	4.6	Sodium chloride (5.8 per cent) 63.5 c.c.
40'–50'	13.4	3.4	intravenously
50'–60'	12.1	4.0	
7. 0'–10'	11.2	4.0	
10'–20'	6.6	4.0	
20'–30'	5.5	4.0	
30'–40'	5.0	5.0	
40'–50'	6.8	4.2	
50'–60'	5 7	2.6	
8. 0'–10'	5.3	3.0	
10'–20'	4.8	3.0	
20'–30'	5 0	2.8	
30'–40'	4.3	3.6	
40'–50'	4.1	3.4	
50'–60'	3.9	3 2	
9. 0'–10'	11.9	54.2	0'0"–1'45"
10'–20'	12.9	28.0	Sodium sulphate (21.4 per cent) 63.5 c.c.
20'–30'	11.7	19.2	intravenously
30'–40'	7.0	14 0	
40'–50'	5.2	10.4	
50'–60'	4.7	10.0	

$$L = \frac{10.1 + 12.3 + 13.4}{4.6 + 4.0 + 4.0} = 2.84 \text{ (sodium chloride)}$$

$$L = \frac{11.9 + 12.9 + 11.7}{4.3 + 4.1 + 3.9} = 2.97 \text{ (sodium sulphate)}$$

TABLE VIII

Piebald bitch, 6.8 kg. Fasted 36 hours before the operation. Narcotized with paraldehyde (8.1 c.c. in 68 c.c. of water).

TIME	LYMPH c.c.	URINE c.c.	NOTICE
12. 0'–10'	2.8	1.2	
10'–20'	2.6	1.4	
20'–30'	2.6	1.4	
30'–40'	3.0	1.4	
40'–50'	2.9	1.6	
50'–60'	3.1	1.4	
1. 0'–10'	10.0	8.8	0'0"–1'30"
10'–20'	13 6	5.0	Sodium chloride (11.6 per cent) 34 c.c.
20'–30'	10.4	4.8	intravenously
30'–40'	9.9	3.8	
40'–50'	7.9	3.2	
50'–60'	5.1	2.2	
2. 0'–10'	5.5	2.0	
10'–20'	6.3	2.0	
20'–30'	5.8	1.6	
30'–40'	5.0	2.4	
40'–50'	4.0	2.2	
50'–60'	3.8	1.4	

$$L = \frac{10.0 + 13.6 + 10.4}{3.0 + 2.9 + 3.1} = 3.77$$

We see that the effect of the second class of lymphagogues is greater in proportion to the osmotic pressure of the solution used. Therefore their action may be said to be a salt action, as Heinz[4] has stated.

Starling[5] and Cohnstein[6] explain the action of the second class of lymphagogues as follows: On the injection of the lymphagogues into the blood, the osmotic pressure of the circulating fluid is largely increased. As a result water is attracted from lymph and tissues into the blood by a process of osmosis until the osmotic pressure of the circulating blood is restored to normal. A condition of hydremic plethora is thereby produced, generally attended by a rise of pressure in the capillaries. This rise of pressure is proportional to the increase of the volume of the blood and, therefore, to the osmotic pressure of the solution injected. This rise of capillary pressure causes a great increase in the transudation of fluid from the capillaries, and consequently an increase in the lymph flow.

To fulfill this condition the vessel wall must be completely semipermeable and the substance injected not allowed to come out of the vessel into the lymph spaces. But as stated by Heidenhain and Cohnstein, the injected substance is found in the lymph in nearly the same amount as in the blood. Moreover, as Starling points out, it is not easy to explain the movement of the water in two opposite directions at the same time, i.e., its coming from the lymph spaces into the vessel to dilute the blood and its passing from the vessel into the lymph spaces by filtration.

I would therefore explain the action of the second class of lymphagogues as follows: A hypertonic solution injected into the blood will be diluted with water attracted into the vessels from the lymph spaces. At the same time the injected substance will be excreted in part by the kidneys and will in part pass into the lymph spaces. Arrived here in a concentrated state, it robs the tissue cells of water by osmosis so that the volume of the interstitial fluid is largely increased and the excess flows away into the lymphatics, thus giving rise to the largely increased lymph flow. Of course the substance in the lymph spaces will also be partly fixed by the tissue cells. Thus we see that the glomeruli and the blood capillaries act for the same purpose of regulating the constitution of the blood. The emigration of the injected substance into the lymph spaces from the blood vessels is not explained by such physical processes as filtration or osmosis. It may be due to the secretory action of the endothelial cells. Accordingly the second class of lymphagogues excites the secretory action of the endothelial cells of both glomeruli and blood capillaries.

I have pointed out that if the osmotic pressures of all the above solutions are equal, the effects upon the increasing lymph flow are the same, but those upon increasing urine output are different. The effect will be the most with sodium sulphate and least with sodium chloride. It is easy to see that sodium chloride is more fixed than sodium sulphate by tissue cells since the former gets more easily into the tissue cells because it is the more diffusible. The less diffusible substance must be more quickly excreted by the kidneys and in greater amount, while the more diffusible substance does not need to be excreted so rapidly, being fixed by tissue cells in greater quantity.

THE FIRST CLASS OF LYMPHAGOGUES

Heidenhain[2] stated that substances of this class exert a specific influence on the endothelial cells, causing them to secrete an increased amount of lymph. Starling[5] explained their action by increased permeability of the capillaries. Asher[3] stated that the first class increases liver function and that increased lymph flow is caused by increased physiologic function of some organ.

No one has tested the action of this class on fluids placed in the body outside the blood vessels. I have observed the influence of peptone on Ringer's solution previously infused into the abdominal cavity, comparing the increase of lymph flow with that of cases in which peptone alone was injected without preliminary infusion of Ringer's solution.

The dose of Ringer's solution was 50 c.c. per kg. of body weight; its temperature was 38° C.

At first the effect of Ringer's solution alone on lymph flow and urine output was observed.

TABLE IX

Yellow bitch, 6.7 kg. Fasted 36 hours before the operation. Narcotized with paraldehyde (8.0 c.c. in 67 c.c. of water).

TIME	LYMPH C.C.	URINE C.C.	NOTICE
1.20′–30′	1.6	0.7	
30′–40′	1.7	0.7	
40′–50′	1.7	0.7	
50′–60′	1.5	0.7	
2. 0′–10′	1.7	0.7	
10′–20′	1.5	0.8	
20′–30′	1.5	0.6	
30′–40′	1.7	0.6	30′–34′ Ringer's solution
40′–50′	2.1	1.0	340 c.c. intraperitoneally
50′–60′	1.9	1.6	
3. 0′–10′	2.2	1.4	
10′–20′	2.4	0.8	
20′–30′	2.4	0.8	
30′–40′	2.2	0.8	
40′–50′	2.3	0.8	
50′–60′	2.3	1.4	
4. 0′–10′	2.3	1.4	
10′–20′	2.5	1.0	
20′–30′	2.6	1.2	
30′–40′	2.5	1.2	
40′–50′	2.9	1.2	
50′–60′	2.9	1.6	
5. 0′–10′	2.7	2.4	
10′–20′	3.0	2.2	
20′–30′	2.5	1.2	
30′–40′	2.5	1.6	
40′–50′	2.5	1.8	
50′–60′	2.5	1.6	

After infusion with Ringer's solution both the lymph flow and the urine output increased slightly and slowly. In 3 other cases the results were the same. On injecting peptone into the blood vessel, the lymph flow increases and the urine output in most cases first decreases then increases again, as shown in Table X.

Tables XI and XII represent 12 experiments; in 7 of which the increase of lymph flow was much more than the controls (Table X and 9 other experiments). In 4 others of the 12, the increase of lymph flow was nearly the same or only a little more than the controls; but the increase in urine output was much more than that in the controls. In only 1 case was neither the lymph flow nor the urine output more than in the controls.

TABLE X

Black bitch, 7.5 kg. Fasted 36 hours before the operation. Narcotized with paraldehyde (9.0 c.c. in 75 c.c. of water).

TIME	LYMPH C.C.	URINE C.C.	NOTICE
11.25′–35′	2.4	1.0	
35′–45′	2.6	1.2	
45′–55′	2.2	1.0	
55′–65′	2.3	1.0	
12. 5′–15′	2.3	1.0	
15′–25′	2.2	1.0	25′–27′ peptone (2 per cent) 37.5 c.c. intravenously
25′–35′	8.3	0.2	
35′–45′	8.7	0.2	
45′–55′	5.7	1.4	
55′–65′	5.8	3.2	
1. 5′–15′	6.1	4.3	
15′–25′	4.3	1.6	
25′–35′	3.1	2.2	
35′–45′	3.2	2.2	
45′–55′	2.5	2.4	

$$L = \frac{8.3 + 8.7 + 5.7}{2.3 + 2.3 + 2.2} = 3.33$$

In the majority of 9 experiments the proportion L was between 2 and 3, the highest being 3.3 and the lowest 1.87.

TABLE XI

Piebald bitch, 6.0 kg. Fasted 36 hours before the operation. Narcotized with paraldehyde (7.2 c.c. in 60 c.c. of water).

TIME	LYMPH C.C.	URINE C.C.	NOTICE
11.45′–55′	1.2	0.6	
55′–65′	1.8	0.6	
12. 5′–15′	1.5	0.4	
15′–25′	1.5	0.4	
25′–35′	1.8	0.4	
35′–45′	1.9	0.4	
45′–55′	1.6	0.4	
55′–65′	1.5	0.4	
1. 5′–15′	1.0	0.4	5′–10′ Ringer's solution 300 c.c. intraperitoneally
15′–25′	1.1	0.4	
25′–35′	1.0	0.4	
1.35′–45′	8.7	0.2	35′–37′ peptone (2 per cent) 30 c.c. intravenously
45′–55′	10.0	0.2	
55′–65′	6.4	1.4	
2. 5′–15′	3.4	1.0	
15′–25′	3.5	1.0	
25′–35′	5.2	0.6	
35′–45′	5.2	0.4	

$$L = \frac{8.7 + 10.0 + 6.4}{1.0 + 1.1 + 1.0} = 8.09$$

Thus we see that peptone intravenously promotes the emigration of Ringer's solution from the abdominal cavity into the lymphatics.

For explanation we may assume the following as the action of peptone: (1) Peptone may act on the fluid in the abdominal cavity so that it can emigrate more easily into the lymphatics. (2) Peptone may act either on the wall of the lymphatics or on that of the blood vessels making it easier for the fluid to migrate into the lymphatic system.

TABLE XII

Yellow bitch, 8.7 kg. Fasted 36 hours before the operation. Narcotized with paraldehyde (10.4 c.c. in 87 c.c. of water).

TIME	LYMPH c.c.	URINE c.c.	NOTICE
1.50′–60′	3.2	0.8	
2. 0′–10′	3.1	0.8	
10′–20′	3.6	0.8	
20′–30′	3.1	1.0	
30′–40′	3.6	1.0	40′–48′ Ringer's solution
40′–50′	2.8	1.2	440 c.c. intraperitoneally
50′–60′	2.7	1.0	
3. 0′–10′	8.0	0.2	0′–2′ peptone (2 per cent) 43 c.c.
10′–20′	5.9	0.5	intravenously
20′–30′	4.5	2.3	
30′–40′	4.4	4.6	
40′–50′	2.8	3.2	
50′–60′	2.5	2.2	
4. 0′–10′	2.3	5.0	
10′–20′	2.5	11.0	
20′–30′	2.4	20.0	
30′–40′	2.3	21.0	
40′–50′	2.2	11.0	
50′–60′	2.5	9.6	
5. 0′–10′	3.0	7.2	
10′–20′	2.8	9.2	

$$L = \frac{8.0 + 5.9 + 4.5}{3.6 + 2.8 + 2.7} = 2.02$$

TABLE XIII

Piebald bitch, 5.5 kg. Fasted 36 hours before the operation. Narcotized with paraldehyde (6.6 c.c. in 55 c.c. of water).

TIME	LYMPH c.c.	URINE c.c.	NOTICE
11.55′–65′	3.0	0.6	
12. 5′–15′	3.0	0.6	
15′–25′	2.8	0.6	
25′–35′	2.9	0.6	
35′–45′	3.0	0.6	
45′–55′	3.3	0.6	
55′–65′	2.7	0.6	
1. 5′–15′	2.6	0.6	7′–11′ Ringer's solution 275 c.c.
15′–25′	2.3	0.6	and peptone (2 per cent) 55 c.c.
25′–35′	2.1	0.6	intraperitoneally
35′–45′	2.1	0.6	
45′–55′	2.3	0.6	
55′–65′	2.0	0.6	
2. 5′–15′	2.6	0.6	
15′–25′	1.9	0.6	
25′–35′	2.4	0.6	

Taking these factors into consideration, I investigated whether or not peptone increases the lymph flow when infused into the abdominal cavity, mixed with the Ringer's solution. (See Table XIII.)

Neither the lymph flow nor the urine output increases, in comparison with cases in which Ringer's solution alone is infused into the abdominal cavity. Although peptone may indirectly facilitate passage of fluid from the abdominal cavity into the lymphatic system, it does not do so by acting directly on this fluid.

Then we must decide whether peptone acts on the wall of the lymph vessel or on that of the blood vessel. If the isotonic fluid injected into the abdominal cavity is absorbed entirely through the blood vessels, as Orlow[7] and Hamburger[8] have stated, peptone must act on the blood vessel so that the fluid once absorbed into the blood vessel passes again into the lymph spaces in greater quantity than before. If the resorption from the abdominal cavity is entirely due to the lymphatic system, as Cohnstein[9] postulates, peptone must act on the lymphatic vessel to promote the passage of the fluid directly into the lymphatic system from the abdominal cavity.

But it is not settled whether resorption from the abdominal cavity is entirely through the lymphatics or entirely through the blood vessels. It may occur rather through both. We have no experimental method for determining the action on the lymphatic system. But if peptone really acts on the blood vessels, an isotonic fluid which has been slowly infused directly into the blood vessels must also pass into the lymph spaces after the injection of peptone. Therefore I injected peptone into the right femoral vein, while at the same time the Ringer's solution was being infused very slowly into the left femoral vein (5 c.c. per kg. of body weight took twenty-five minutes). According to Orlow,[7] the resorption of an isotonic fluid from the abdominal cavity is not rapid, 1.7-2.0 c.c. being absorbed in an hour when 25-33 c.c. per kg. has been

Table XIV

Black bitch, 9.4 kg. Fasted 36 hours before the operation. Narcotized with paraldehyde (11.3 c.c. in 94 c.c. of water).

TIME	LYMPH C.C.	URINE C.C.	NOTICE
12.25′–35′	2.8	1.2	
35′–45′	2.9	1.2	
45′–55′	3.2	1.4	
55′–65′	3.0	1.2	0′–25′ Ringer's solution 47 c.c.
1. 5′–15′	4.5	1.2	in the left femoral vein.
15′–25′	8.1	1.0	
25′–35′	6.8	2.6	5′–7′ peptone (2 per cent) 47 c.c.
35′–45′	5.0	2.0	in the right femoral vein
45′–55′	3.9	1.4	
55′–65′	3.2	1.0	
2. 5′–15′	2.6	1.2	
15′–25′	2.6	1.2	
25′–35′	2.4	1.2	
35′–45′	2.3	1.0	
45′–55′	2.7	1.0	

$$L = \frac{4.5 + 8.1 + 6.8}{2.9 + 3.2 + 3.0} = 2.13$$

injected. Therefore not so much Ringer's solution was infused into the left femoral vein.

In this protocol (Table XIV) we see that the increase of lymph flow is not more than when peptone alone is injected into the vein. In 4 other experiments the results were nearly the same. Accordingly the action of peptone is not on the blood vessels. Thus we may conclude that peptone promotes the migration of the fluid from the abdominal cavity into the lymphatics, acting on their walls, unless it acts on the fluid after it has itself been altered in the body. We cannot decide here in which way peptone acts. But as peptone promotes the passage of fluid from the abdominal cavity it is easy to see that the migration of tissue fluid will also be promoted by peptone. This we assume is the cause of the increase of lymph flow by peptone injection.

I have mentioned before that in 4 out of 12 cases in which Ringer's solution had been previously injected into the abdominal cavity and then peptone injected, the lymph flow was not more, but that the urine output was much more, than in the controls. The great increasing of urine output in these 4 cases shows that the infused fluid went for the most part into the blood circulation and was excreted by the kidney. It seems that in these 4 animals resorption from the abdominal cavity was mainly through the blood vessels, with the result that lymph flow was not greater than in the controls.

It is well known that peptone injection increases the concentration of the blood. Underhill and Ringer[10] explained this phenomenon as follows: On injection of peptone, the permeability of blood capillaries increases, as Starling stated; and consequently, because of this increased permeability, more plasma goes into the lymph spaces, and the blood is more concentrated. But this might be a secondary phenomenon to the primary passage of tissue fluids into the lymphatic system.

PITUITRIN

Numerous experiments on the influence of hypophyseal extract on the urine output have been carried out. The current opinion accepts its being first antidiuretic and then diuretic; yet divergences exist as to the cause of the diuresis.

Hoskins and Means[11] state that pituitrin excites secretion of the renal cells. King and Stoland[12] insist on dilatation of the kidney vessels. Pentimali and Querica[13] explain the diuresis from the point of blood pressure overcoming the constriction of kidney vessels and explain antidiuresis by the preliminary predominance of the latter. Veil,[14] Meyer and Meyer Bisch[15] claim that pituitrin concentrates the tissue fluids. The water liberated by the concentration passes into the blood vessels and is excreted through the kidneys. Especially Meyer and Meyer Bisch[15] insist on the concentration of the tissues, since they have demonstrated that on injection of pituitrin, a diminution of lymph flow and the presence of a more concentrated lymph result. Oehme[16] claims that concentration of the tissues cannot be proved, and according to his experiments pituitrin diuresis must be due to stimulation of the renal cells. Bauer and Aschner[17] also deny the primary action of pituitrin on the tissues. Using the blood concentration as a standard for comparison, Underhill and Pack[18] found the pituitrin affected kidney less sensitive than the normal kidney to the hydremic stimulus; hence pituitrin is not diuretic but relatively antidiuretic. They state further that renal ligature does not modify the production of hydremia by pituitrin and that as pituitrin inhibits water diuresis the antidiuretic action and the hydremia are probably due to some alteration

of capillary permeability. Momose[19] asserts that on pituitrin injection the lymph flow and the blood concentration decrease and that the urine output first decreases but after a few minutes increases. Fromherz[20] believes that pituitrin acts in two phases, antidiuretic and diuretic. The duration of each phase differs according to the individual, so that pituitrin appears at times antidiuretic and at other times diuretic.

The effect of pituitrin on lymph flow may shed further light on its antidiuretic and diuretic action. The drug used was *pituitrin* (Parke, Davis & Co.), the dosage 0.2 gram per kg. body weight. The methods of measuring the lymph flow and the urine output were as described before. To know the concentration of blood, the hemoglobin content was determined after Sahli's method. The blood was always obtained from clean-cut incisions in the marginal ear veins of dogs.

TABLE XV

Piebald bitch, 5.5 kg. *Fasted 36 hours before the operation. Narcotized with paraldehyde* (6.6 c.c. in 55 c.c. of water).

TIME	LYMPH C.C.	URINE C.C.	HEMOGLOBIN	NOTICE
12.48′–58′	3.2	0.8		
58′–68′	3.6	0.6	53	
1. 8′–18′	3.6	0.6	53	
18′–28′	3.1	0.6	52	
28′–38′	3.9	1.0	52	
38′–48′	3.2	1.0	54	
48′–58′	3.4	1.0	54	
58′–68′	1.8	5.0	54	58′0″–58′30″ pituitrin
2. 8′–18′	1.1	3.2		1.1 c.c. intravenously
18′–28′	1.7	1.2	52	
28′–38′	2.4	1.2		
38′–48′	2.7	0.8	50	
48′–58′	2.3	0.8		
58′–68′	2.3	1.0	52	
3. 8′–18′	2.6	1.6		
18′–28′	2.9	1.0	52	
28′–38′	3.2	1.2		
38′–48′	2.2	0.8	54	
48′–58′	2.7	1.0		
58′–68′	2.7	1.2	54	
4. 8′–18′	1.7	1.4		
18′–28′	2.1	1.0	54	

The results of the intravenous injection of pituitrin are given in Table XV. We see that the lymph flow and the blood concentration decrease but that the urine output increases. We cannot see the first antidiuretic period; this is probably because the catheter was retained in the bladder; if the catheter had been in the ureter (Momose[19]), the antidiuretic period would have been perceived.

The results of the subcutaneous injection of pituitrin are given in Table XVI.

The lymph flow and the blood concentration decrease, but there is no increase of urine output.

The results of infusion with Gum-Ringer solution are given in Table XVII. The Gum-Ringer solution contains 2 per cent gum arabic in Ringer's solution for the purpose of keeping it within the vessel as long as possible. In Table XVII the results from infusion of this solution alone were observed as the

TABLE XVI

Piebald bitch, 6.5 kg. Fasted 36 hours before the operation. Narcotized with paraldehyde (7.8 c.c. in 65 c.c. of water).

TIME	LYMPH c.c.	URINE c.c.	HEMOGLOBIN	NOTICE
1. 5′–15′	4.3	1.1		
15′–25′	4.1	1.0	72	
25′–35′	3.6	1.2	73	
35′–45′	3.2	1.0		
45′–55′	3.1	1.0	72	
55′–65′	2.1	1.0		55′0″–55′45″ pituitrin
2. 5′–15′	2.4	1.2	73	1.3 c.c. *subcutaneously*
15′–25′	1.4	1.4	70	
25′–35′	0.7	1.2		
35′–45′	0.8	1.4	66	
45′–55′	1.2	1.4	66	
55′–65′	3.7	1.6		
3. 5′–15′	4.0	1.4	68	
15′–25′	3.2	1.3		
25′–35′	3.8	1.4	71	
35′–45′	2.3	1.6	71	
45′–55′	2.4	1.2	70	
55′–65′	2.8	1.4	70	
4. 5′–15′	2.9	1.3	74	
15′–25′	2.5	1.4	73	
25′–35′	3.2	1.8	75	
35′–45′	3.2	2.0		
45′–55′	2.6	1.8		

TABLE XVII

Yellow bitch, 4.2 kg. Fasted 36 hours before the operation. Narcotized with paraldehyde (5.0 c.c. in 42 c.c. of water).

TIME	LYMPH c.c.	URINE c.c.	HEMOGLOBIN	NOTICE
1.58′–68′	3.4	0.4	50	
2. 8′–18′	2.8	0.8	50	
18′–28′	2.8	0.6	50	
28′–38′	2.8	0.4	50	
38′–48′	8.2	0.4		38′–55′ Gum-Ringer solution
48′–58′	10.5	1.0	47	63 c.c. intravenously
58′–68′	7.4	0.6	45	
3. 8′–18′	4.0	0.4	47	
18′–28′	3.7	0.8	50	
28′–38′	3.7	0.6		
38′–48′	2.1	1.0	50	
48′–58′	2.5	1.0		
58′–68′	2.5	0.4	50	

control. This infusion causes a diminution in blood concentration and an increase in lymph flow but no change in urine output.

In Tables XVI to XVIII I would draw attention to the fact that while pituitrin alone subcutaneously, or Gum-Ringer solution alone intravenously, do not cause an increased urine output, their *simultaneous* administration, by the same routes, does produce an increase in urine flow. The blood concentration is more decreased, while the lymph flow increases much less than the Gum-Ringer control. Thus we see that the urine output increases after intravenous injection (Table XV), but does not after subcutaneous injection of pituitrin (Table XVI), while the lymph flow decreases in both cases. Therefore it is evident that the increase in urine output is not the cause of lymph

TABLE XVIII

Piebald bitch, 4.3 kg. Fasted 36 hours before the operation. Narcotized with paraldehyde (5.1 c.c. in 43 c.c. of water).

TIME	LYMPH C.C.	URINE C.C.	HEMOGLOBIN	NOTICE
3.58′–68′	2.8	1.0	52	
4. 8′–18′	2.7	1.0	52	
18′–28′	2.9	1.0	52	
28′–38′	2.3	1.0	52	37′0″–37′30″ pituitrin
38′–48′	4.0	0.4		0.86 c.c. subcuaneously
48′–58′	6.3	3.4	46	
58′–68′	5.9	3.8	46	38′–53′ Gum-Ringer solution
5. 8′–18′	5.7	2.6	48	64.5 c.c. intravenously
18′–28′	3.8	1.6	50	
28′–38′	3.4	1.2	48	
38′–48′	3.3	0.8	47	
48′–58′	3.2	0.6	48	
58′–68′	3.5	0.6	51	
6. 8′–18′	3.2	0.6	52	
18′–28′	2.9	0.6		
28′–38′	2.9	0.6	52	

diminution. Since, however, the intravenous injection of pituitrin causes an hydremic plethora and at the outset an antidiuresis, it is probable that pituitrin inhibits the secretory action of endothelial cells of the blood capillaries and of the kidney glomeruli. The diminution of lymph flow is due to inhibition of secretion by the capillary endothelium. Because of the inhibiting action on both glomeruli and blood capillaries, the hydremia will be more and more heightened after the injection of pituitrin; and when the hydremic stimulus overcomes the inhibiting action of pituitrin, the period of antidiuresis changes to that of diuresis. This is the reason why pituitrin is both antidiuretic and diuretic.

The stimulus of the hydremia caused by subcutaneous injection of pituitrin alone is not enough to overcome the inhibiting action, but if the Gum-Ringer solution is supplied at the same time and the hydremia thus increased, the inhibition no longer dominates. The insistence of Underhill and Pack[18] that on injection of pituitrin the increased urine output is very slight compared to the marked diminution of blood concentration, also explains the inhibiting action on the secretion of both blood capillaries and glomeruli.

SUMMARY AND CONCLUSION

1. The second class of lymphagogues stimulates the secretory activity of endothelial cells of both blood capillaries and glomeruli. Since both are stimulated not only the lymph flow but also the urine output is markedly increased.

2. *The first class of lymphagogues promotes the migration* of tissue fluids into the lymphatic system. The urine output increases owing to the passage of the tissue fluids into the circulation through the lymphatics. It does not increase so much following the second class which stimulates secretion in the kidney.

3. Pituitrin inhibits the secretion of endothelial cells of both blood capillaries and glomeruli. Therefore the lymph flow, the blood concentration, and

the urine output decrease; but the urine output increases soon after the stimulus of the hydremia is heightened.

It is probable that even in the normal state such substances, which act like the lymphagogues, appear in the lymph spaces as products of metabolism. Some of them increase the tissue fluids, attracting water from the tissue cells and the blood, and others promote the migration of the tissue fluids into the lymphatics. Accordingly, the lymph flowing out of the thoracic duct may contain the excretions produced by the metabolism of tissue cells. To this extent, the cellular physiologic theory may be true, but that the lymphagogues themselves increase the functions of organs, is a theory with objectionable points.

Thus the lymphatic system belongs to the excreting system and it plays a great part in regulating the constitution of the blood. An abnormal, either qualitative or quantitative substance, existing in the blood, must be excreted on one hand by the kidney and on the other by the blood capillaries into the lymph spaces where part of it will be fixed by the tissue cells and part goes farther into the lymphatics. It returns again into the circulation through the lymphatic system to be excreted finally through the kidney.

Thus the abnormal substance will be in part conserved in the lymphatic system and in the tissue cells for a while, in order that the kidney will not be burdened too much at one time. In this sense we may call the tissues the "forekidney."

The blood capillaries act to the same purpose as the glomeruli, the latter carrying the catabolic substance out of the body, the former sending it into the lymph spaces. From this statement the blood capillaries may be called "pre-glomeruli."

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THE ANTIKETOGENIC INFLUENCE OF INSULIN IN DIABETES MELLITUS*

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BANTING, Campbell, and Fletcher[1] have observed the disappearance of ketone bodies from the urine of diabetic patients four hours after the administration of insulin and their reappearance in eight hours. After repeated large doses of insulin Campbell[2] found a decrease of the ketone bodies of the blood and urine with an increase of the carbon dioxide combining power of the blood plasma and an increase in the tension of carbon dioxide of the alveolar air. In these data, however, the level of the carbon dioxide combining power is not always dependent upon changes in the concentration of the ketone bodies of the blood or urine. Rabinowitch[3] obtained a fall of the blood ketone bodies and a rise in the carbon dioxide combining power after insulin, but he concludes that the rise in the carbon dioxide combining power is not proportional to the drop of blood ketone bodies. These reports demonstrate the efficacy of insulin therapy in diabetes; however, their value in demonstrating its antiketogenic action is open to question, since other well-known antiketogenic agents such as glucose, saline solutions, and alkalies were utilized to relieve the acidosis.

The object of the present study was to determine the immediate action of insulin upon the production and excretion of the ketone bodies and upon the acid base equilibrium of the blood in human cases of diabetes mellitus. In order to eliminate the dietetic factor the subjects were starved for twelve to fourteen hours previous to the administration of the insulin. Also, during the period of observation (from eight to ten hours), food was withheld, but the drinking of water was permitted *ad libitum*. As a control on the effect of the starvation itself upon the blood, the blood sugar and carbon dioxide combining power were determined in five cases at two hourly intervals for eight to ten hours under similar conditions of food privation and both with and without insulin. Starvation without insulin continuously decreased the blood sugar. The lowest level reached was about 71 per cent of the original concentration. The drop in blood sugar after the insulin was, however, much greater, a level of 50 to 30 per cent of the original concentration was obtained. This decrease reached its maximum in four to six hours and was followed by an appreciable rise. In 3 instances the carbon dioxide combining power rose steadily during starvation, but in the 2 remaining cases a continuous fall was noted. On the contrary, after the insulin in all of the five cases a rise in the carbon dioxide combining power was noted for six hours with subsequently a slight drop. Evidently the restriction of food was a negligible quantity in influencing the results to be discussed.

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Since it was planned to study the antiketogenic effect of insulin alone, it was essential to avoid the development of hypoglycemic shock necessitating the use of emergency measures. In an extensive series of preliminary experiments it was found that one unit of insulin would decrease the blood sugar of an individual weighing from 50 to 70 kg. about 0.008 per cent in four to six hours. Upon the basis of this observation, the dose of insulin was graded to obtain the maximum beneficial effect without producing untoward results. The units of insulin employed in the following experiments were the quantities calculated to reduce the blood sugar to about 0.150 per cent, and it was the only therapeutic agent utilized. A control specimen of blood was obtained before and after the injection of the insulin, at one- or two-hour intervals, until its influence upon the sugar and carbon dioxide combining power had vanished. Specimens of urine were also collected at corresponding intervals and preserved with toluene until analyzed. The control specimen of urine represented the collection over a one- or two-hour period before the insulin. The insulin was injected subcutaneously. The specimens of blood were analyzed immediately after withdrawal by venipuncture to avoid any source of error due to glycolysis or to production of fixed acid bodies in the blood upon standing. The blood sugar was determined by the Folin-Wu technic and the carbon dioxide combining power by the Van Slyke method. Van Slyke and Fitz's[4] gravimetric method was employed for the ketone bodies of the blood, and the procedure described by Van Slyke[5] was used for the urine. In the blood the total ketone bodies were calculated as acetone in milligrams per 100 c.c., and in the urine the total ketone bodies are reported as acetone in concentration per cent, and as grams excreted during the interval. The urine sugar was estimated by Benedict's most recent method,[6] and where the amount exceeded 0.5 per cent, parallel determinations were made with the same author's volumetric method.[7]

DISCUSSION OF RESULTS

In Table I are presented the results obtained in 10 series of observations upon 7 cases of diabetes. In the first 2 of these, analyses were confined to the blood, since it was impossible to obtain samples of urine at the proper intervals from these patients who at the outset were in coma. It will be noted that in all instances there is a prompt decrease in the blood sugar reaching a minimum concentration of about 50 per cent of the control figure. The lowest value obtained was 0.096 in Case 10. The control specimen of blood from this patient showed a pronounced lipemia and since it was desired to observe the influence of the insulin on the blood lipoids, an unusually large dose was administered in this case.* In no instance was a hypoglycemia produced. It is worthy of note that in but five of the ten cases was the calculated effect of the insulin upon the sugar obtained.

More striking, however, are the changes observed in the urine sugar. During the control periods the urines contained from 1.06 to 7.8 gm. of sugar, but after the insulin administration it was rapidly reduced to well within nor-

*A more complete report of this case has been made by Lough and Killian, Medical Clinics of North America, July, 1924, viii, 337.

TABLE I

CHEMICAL CHANGES IN BLOOD AND URINE AFTER INSULIN

CASE	TIME	BLOOD			URINE					REMARKS
		SUGAR	CO_2 C. P.	KETONE BODIES	VOL. C.C.	SUGAR		KETONE BODIES		
		PER CENT	VOL. PER CENT	MG. PER 100 C.C.		PER CENT	GM.	PER CENT	GM.	
1—L. C.	2.30 P.M.	0.514	12.6	87.8						Blood plasma PH = 7.13
Age = 28—M.	3.30 "	Insulin	40 units							
6/5/23	5.30 "	0.312	26.3	60.4						
	7.30 "	0.240	32.8	44.8						
	9.30 "	0.300	39.3	29.8						
	11.30 "	0.308	38.4	29.8						
	12.00 "	Insulin	15 units							
6/6/23	8.15 A.M.	0.254	37.4	27.5						Blood plasma PH = 7.28
2—A. W.	10.55 P.M.	0.468	20.4	54						
Age = 39—F.	11.00 "	Insulin	20 units							
7/31/23	1.00 A.M.	0.364	22.1	39.8						
8/ 1/23	3.00 "	0.332	25.8	30.6						
	5.00 "	0.384	28.1	25.2						
	6.00 "	Insulin	20 units							
	1.00 P.M.	0.306	34.3	15.0						
3—N. L.	8.20 A.M.	0.308	32.8		197	2.50	4.92	0.62	1.22	Urine collected 8.20-9.30 A.M.
Age = 36—M.	9.30 "	Insulin	20 units							
1/6/23	10.30 "	0.262	34.7		100	2.63	2.63	0.45	0.45	
	11.30 "	0.222	41.4		75	1.0	0.75	0.24	0.18	
	12.30 P.M.	0.178	42.4		34	0.74	0.25	0.15	0.052	
	1.30 "	0.158	46.2		110	0.02	0.022	0.055	0.061	
	2.30 "	0.151	44.3		125	0.05	0.062	0.044	0.055	
	3.30 "	0.172	44.3							
	4.30 "	0.192	41.4		163	0.03	0.048	0.051	0.084	

TABLE 1—CONT'D

CASE	TIME	BLOOD			URINE					REMARKS
		SUGAR PER CENT	CO_2 C. P. VOL. PER CENT	KETONE BODIES MG. PER 100 C.C.	VOL. C.C.	SUGAR PER CENT	SUGAR GM.	KETONE BODIES PER CENT	KETONE BODIES GM.	
4—L. C.	9.00 A.M.	0.342	30.9		250	0.80	2.0	0.76	1.90	Urine collected 7.15-9.15 A.M.
Age = 28—M.	9.15 "	Insulin	30 units							
3/9/23	11.15 "	0.197	35.7		29	0.46	0.13	0.65	0.19	
	1.15 P.M.	0.180	38.5		66	0.01	0.006	0.40	0.266	
	3.15 "	0.131	40.4		65	0.01	0.006	0.15	0.104	
	5.15 "	0.104	44.3		42	0.01	0.004	0.14	0.060	
3/10/23	12.30 A.M.	0.255	36.6							
5—F. L.	8.55 A.M.	0.226	35.3		180	3.2	5.76	0.41	0.748	Urine collected 7.00-9.00 A.M.
Age = 34—M.	9.00 "	Insulin	11 units							
7/23/23	11.00 "	0.226	38.1		75	3.0	2.25	0.22	0.174	
	1.00 P.M.	0.168	33.4		345	0.34	1.20	0.042	0.148	
	3.00 "	0.178	33.4		35	2.2	0.77	0.19	0.067	
	5.00 "	0.254	32.4		105	2.1	2.20	0.22	0.231	
6—A. Y.	10.00 A.M.	0.302	36.6		210	3.5	7.35	0.36	0.760	Urine collected 9.10-10.10 A.M.
Age = 20—M.	10.10 "	Insulin	22 units							
1/10/23	11.10 "	0.274	40.4		65	3.0	1.95	0.45	0.291	
	12.10 P.M.	0.220	52.8		30	1.5	0.45	0.33	0.100	
	1.10 "				305	0.01	0.03	0.018	0.055	
	2.10 "	0.184	56.7		255	0.01	0.02	0.011	0.029	
	3.15 "	0.193	51.9		45	0.11	0.04	0.18	0.081	
	4.15 "	0.157	51.0		115	0.08	0.09	0.054	0.063	
	5.10 "	0.190	51.0		35	0.19	0.09	0.28	0.098	

TABLE I—CONT'D

CASE	TIME	BLOOD			URINE					REMARKS
		SUGAR PER CENT	CO_2 C. P. VOL. PER CENT	KETONE BODIES MG. PER 100 C.C.	VOL. C.C.	SUGAR		KETONE BODIES		
						PER CENT	GM.	PER CENT	GM.	
7—S. G.	4.00 P.M.	0.288	22.3	60.3	285	2.5	7.12	1.07	3.05	Urine collected 3.30-5.30 P.M.
Age = 22—M.	5.30 "	Insulin	22 units							Blood plasma PH = 7.24
3/29/23	7.30 "	0.205	23.3	51.2	265	1.4	3.65	0.472	1.25	
	9.30 "	0.182	28.1	46.0	195	0.18	0.35	0.115	0.224	Blood plasma PH = 7.34
	11.30 "	0.151	32.8	43.9						
3/30/23	1.30 A.M.	0.176	30.0	45.5	120	0.18	0.22	0.13	0.156	
	8.30 "	0.267	30.0	53.4	225	0.40	0.90	0.50	1.125	
8—A. W.	10.00 A.M.	0.282	24.9	45.3	230	2.3	5.29	0.32	0.743	Urine collected 8.20-10.20 A.M.
Age = 51—M.	10.20 "	Insulin	20 units							
7/6/23	12.20 P.M.	0.280	26.8	45.6	225	2.8	6.30	0.29	0.657	
	2.20 "	0.254	29.6	30.2						
	4.20 "	0.274	26.8	26.6	185	2.3	4.25	0.14	0.259	
	5.00 "	Insulin	20 units							
	6.20 "	0.264	32.4	19.6	175	2.1	3.67	0.097	0.171	
9—N. L.	9.00 A.M.	0.308	46.2	32.9	88	1.7	1.47	0.30	0.260	Urine collected 9.00-10.00 A.M.
Age = 36—M.	10.00 "	Insulin	23 units							
1/12/23	11.05 "	0.256	51.9	32.0	56	1.1	0.61	0.18	0.101	" " 10.30-11.00 "
	12.05 P.M.	0.220	57.6	13.0	24	0.28	0.067	0.066	0.016	" " 11.30-12.30 P.M.
	1.06 "	0.180	60.5	2.8	222	0.02	0.044	0.014	0.033	" " 12.30- 1.30 "
	2.12 "	0.150	59.5	14.0	470	0.01	0.047	0.004	0.020	" " 1.30- 2.30 "
	3.05 "	0.172	59.5	14.0	91	0.04	0.036	0.030	0.028	" " 2.30- 3.30 "
	4.08 "	0.190	57.6	15.1	140	0.02	0.028	0.027	0.038	" " 3.30- 4.30 "
	5.08 "	0.181	54.8	17.0	150	0.02	0.030	0.027	0.041	" " 4.30- 5.28 "
10—A. Y.	3.30 P.M.	0.300	41.8	31.0	260	3.0	7.80	0.26	0.681	Urine collected 3.00-5.00 P.M.
Age = 21—M.	5.00 "	Insulin	35 units							
12/15/23	7.00 "	0.134	42.8	29.6	360	0.55	1.98	0.14	0.504	
	9.00 "	0.122	49.4	20.9	150	0.17	0.25	0.19	0.288	
	10.00 "	0.096	53.2	16.0	210	0.04	0.084	0.03	0.063	

mal limits. The lowest level reached by the urine sugar in each case was about 0.5 per cent of the control figure and is *synchronous with the minimum* concentration of the blood sugar.

The study of *changes in the ketone bodies of the blood and urine* in conjunction with the alkali reserve of the blood plasma after insulin reveals a very significant effect of the substance. In the first two cases the administration of insulin resulted in a continuous decrease of the blood ketone bodies with a steady rise in the carbon dioxide combining power of the plasma. Van Slyke and Fitz have placed the normal concentration of *total* ketone bodies as acetone between 1.3 and 2.6 mg. per 100 c.c. Subject 1 showed a drop in the ketone bodies of the blood from 87.8 mg. to 29.8 mg. per 100 c.c. during the six hours following a dose of 40 units of insulin. During the same period the carbon dioxide capacity of the blood plasma rose from 12.6 to 39.3 volumes per cent. Eight hours after a second dose of 15 units, the ketone bodies had fallen to 27.5 mg., and the carbon dioxide combining power rose to 37.4 volumes per cent. It is worthy of note that *the minimum concentration of ketone* bodies corresponds in time with the maximum carbon dioxide capacity of the blood plasma. A similar effect is observed in Case 2 in which the ketone bodies are diminished from 54 to 25.2 mg., paralleled by a rise in the carbon dioxide combining power of the blood plasma from 20.4 to 28.1 volumes per cent in six hours. Here again a second dose of 15 units brought the carbon dioxide capacity up to 34.3 volumes per cent, and the ketone bodies were decreased to 15 mg. per 100 c.c.

In the following 4 cases the excretion of the ketone bodies of the urine has been studied in association with the sugar and carbon dioxide combining power of the blood. All of the data indicate a prompt and continuous decrease in the ketonuria after insulin. It is evident that although the concentration of these compounds in the urine varies with the volume of urine, the actual output is independent of the volume excreted. There is no evidence of the *initial rise and the subsequent fall in the ketonuria, noted by Mosenthal and* Killian,[8] after sodium bicarbonate administration. The maximum excretion of ketone bodies in the urine of normal men under usual conditions reported by Van Slyke is about 0.42 gm. per liter calculated as acetone, i.e., 0.042 per cent. All of the subjects reported in the table showed markedly increased concentration of urine ketone bodies during the control period, from 0.75 to 1.90 gm., i.e., 0.41 to 0.76 per cent. The average decrease was to 5 to 10 per cent of the control figure, to 0.067 to 0.029 gm. (0.19 to 0.011 per cent). This minimum concentration of urinary ketone bodies is encountered at about the time the carbon dioxide combining power has reached its maximum. Subject 5, however, shows *a lag in the decrease of the ketonuria and hyperglycemia.* Here the carbon dioxide capacity rose but 2.8 volumes per cent and that during the first two hours. In this instance the diabetes was complicated by other pathologic conditions.

In the final cases, from 7 to 10 inclusive, concurrent determinations of both the ketone bodies of the blood and urine have been made. The control specimens of blood showed ketone bodies varying from 31 to 60.3 mg. per 100 c.c., whereas the initial urine specimens contained from 0.267 to 3.05 gm.

of these compounds. Following the administration of the insulin there is a continuous decrease in the blood ketone bodies; this drop parallels the rise in the carbon dioxide combining power of the plasma. In Case 9 an unusual fall of the blood ketones from 32.9 to 2.8 mg. per 100 c.c. was noted, but in the other instances the average decrease is but 50 per cent of the initial concentration. A simultaneous but far more striking decrease of the ketone bodies of the urine is seen, the average reaching 10 to 30 per cent of the initial figure. As observed above for the urine sugar, the effect of the insulin upon the ketone bodies of the urine is more pronounced than upon the ketone bodies of the blood. It is of interest to note that in Subjects 1, 2, and 8 the influence of the insulin upon the ketosis is magnified by the administration of a second dose after the maximum effect of the first dose has been obtained. In the remaining cases, a rise of the sugar and ketone bodies of the blood and urine and a decrease of the carbon dioxide combining power of the blood plasma appear six to eight hours after the insulin. The rise of the ketone bodies, however, follows the increase of the sugar.

It is evident from the data presented that the decrease of the ketone bodies, both of the blood and urine, is accompanied by a rise in the alkali reserve of the blood plasma. On the basis of the statement of Palmer and Van Slyke[9] that 0.5 gm. of sodium bicarbonate will raise the carbon dioxide combining power one volume per cent for each forty-two pounds of body weight, it has been calculated from the results recorded that one unit of insulin is equivalent in its influence upon the alkali reserve of the blood to about 0.7 gm. of sodium bicarbonate taken orally. Such a comparison is, however, but a rough approximation, since all of the subjects studied manifested diabetic ketosis. In such conditions, as Palmer and Van Slyke state, the organism is forming acid at a rapid rate; hence the sodium bicarbonate will elevate the plasma bicarbonate by less than the calculated amount because part of the alkali given is neutralized by the acids formed during the necessarily gradual administration.

Determinations of the plasma P_H were made by the method of Myers, Schmitz, and Booher[10] in two subjects (Cases 1 and 7). In the first instance the plasma P_H was 7.13 with a plasma CO_2 capacity of 12.6 volumes per cent. After the administration of 55 units of insulin in two doses, the CO_2 capacity of the plasma had reached 37.4 volumes per cent with a P_H of 7.28. Again in Case 7 the control blood plasma showed a CO_2 capacity of 22.3 per cent and a P_H of 7.24. Four hours later the CO_2 capacity had risen to 28.1 and the P_H to 7.34. These findings indicate that insulin induces a return towards normal of both the carbon dioxide combining power and of the P_H of the plasma. These observations are in keeping with the statement of Cullen and Jonas[11] that under insulin treatment the alkali reserve and the P_H of the blood plasma return to their normal level coincidently, and there appears to be a consistent relation between these two factors during their return to normal.

It is apparent that the improvement in the alkali reserve of the blood plasma is dependent upon the inhibition of the ketogenesis. Corresponding to the decrease in the ketosis there is noted a drop in the hyperglycemia and glycosuria. Ringer[12] has reported in phlorizinized dogs a rise in the respiratory

quotient after insulin and an enhanced ability to metabolize glucose. This author found that in the experimental animals one unit of insulin would effect the complete oxidation of 2 gm. of glucose per hour. Briggs, Koechig, Doisy, and Weber[13] have shown that under the influence of insulin the liver of phlorizinized dogs regains the ability to convert glucose into lactic acid and subsequently to dispose of this by oxidation. Dudley and Marrian[14] report that the disappearance of sugar from the blood after insulin cannot be attributed to an increased storage of glycogen in liver or muscles. Lyman, Nicholls, and McCann[15] have demonstrated, moreover, a rise in the respiratory quotients and of the heat production in normal human subjects after insulin; hence it is logical to attribute the diminution of the hyperglycemia and the glycosuria of diabetes to an increased oxidation of glucose stimulated by the insulin. A more complete oxidation of the fatty acids would thus be made possible, resulting in a diminution of the ketone bodies. The antiketogenic influence of insulin in diabetes presumably is due to this sequence of events.

SUMMARY

1. The administration of insulin to cases of diabetes mellitus results in an immediate decrease of the ketone bodies of the blood and urine, reaching the maximum in four to six hours.

2. The decrease in the ketosis is accompanied by a corresponding rise in the alkali reserve of the blood plasma.

3. The rise in the carbon dioxide capacity is associated with a proportional increase in the blood plasma P_H.

4. The antiketogenic influence of the insulin apparently is due to the more complete oxidation of the carbohydrates stimulated by this substance.

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GALACTOSE TOLERANCE IN LATENT TETANY*

By Max Erdheim, M.D., Brooklyn, N. Y.

DISTURBANCES of carbohydrate metabolism are almost constant in dogs in which tetany has been induced by the removal of the parathyroid glands. According to Falta,[1] the assimilation limits for dextrose in parathyroidectomized dogs are regularly reduced. Eppinger, Falta, and Rudinger[2] observed a lasting glycosuria in a completely parathyroidectomized dog sustained on a diet not very rich in starchy material. Falta and Rudinger[3] found that the injection of adrenalin into such animals brought about a marked glycosuria. In this connection one may mention the alimentary glycosuria observed in thyroparathyroidectomized dogs by Falkenberg[4] and by Underhill and Tadasu-Saiki;[5] these authors, however, considered this phenomenon to be the result of a hypofunction of the thyroid gland. In brief, many observations are in accord to the effect that parathyroidectomy in dogs produces, in association with the artificial tetany, definite and constant abnormalities of carbohydrate metabolism.

In cases of human tetany, disturbances of carbohydrate metabolism similar to those in parathyroidectomized dogs are not commonly observed. Falta and Kahn[6] could not demonstrate such a corresponding decreased tolerance for dextrose in human cases. Only in one case, an acute attack of tetany complicating Graves' disease, did they observe alimentary glycosuria after the acute phase had passed; but they were unable to provoke it again, although they made several attempts with that end in view. Even in this case the objection might well be raised that they were really dealing with a period of glycosuria accompanying Graves' disease, for this complication is rather common in that condition. In view of the striking changes in the carbohydrate metabolism observed in dogs with artificial tetany, it seemed advisable to investigate more fully human cases with reference to the possible existence of similar conditions. If such changes could likewise be demonstrated in human cases, it is obvious that a knowledge of them would be of definite clinical value, since it would furnish the basis for a convenient laboratory test to aid in the recognition of latent tetany.

In this investigation galactose was the sugar chosen for the tolerance tests. The average tolerance for this carbohydrate has been carefully worked out. Rowe[7] demonstrated that the tolerance dose of galactose is 30 gm. for men and 40 gm. for women. The usual amount of galactose given in my tests was 40 gm., although this was not always the case. The dosage of galactose per kilogram of body weight was computed, and these figures are expressed in the tabulation. The blood-sugar level was estimated before the intake of the galactose and at half-hour intervals thereafter until its return to the pre-

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TABLE I

GALACTOSE TOLERANCE IN FIVE CASES OF OUTSPOKEN TETANY

CASE	SEX	AGE	WEIGHT IN KG.	GM. OF GALACTOSE BY MOUTH		BEFORE GALACTOSE INTAKE	PERCENTAGE OF BLOOD SUGAR AFTER GALACTOSE INTAKE					GM. SUGAR IN URINE	ELECTRICAL TESTS MILLIAMPERES		
				ABSOLUTE	PER KG. BODY WEIGHT		½ H.	1 H.	1½ H.	2 H.	2½ H.		KCC	ACC	AOC
1	M	18	37.0	40.0	1.08	0.12	0.15	0.11	0.11	0.11		2.50	0.6	1.4	1.0
2	M	21	60.9	40.0	0.65	0.11	0.15	0.14	0.12	0.10	0.10	0.40	0.8	1.9	1.8
3	M	20	50.0	34.0	0.68	0.12	0.14	0.14	0.11	0.10		0.15	0.9	1.9	2.0
4	M	50	59.5	40.5	0.68	0.10	0.13	0.16	0.11	0.10	0.10	1.80			
5	M	18	48.0	32.6	0.68	0.11	0.14	0.11	0.10	0.11		0.80	0.7	1.6	1.5
Average		25	51.0	37.4	0.75	0.11	0.14	0.13	0.11	0.10	0.10	1.13			

TABLE II

GALACTOSE TOLERANCE IN SEVEN CONTROL CASES

CASE	SEX	AGE	WEIGHT IN KG.	GM. GALACTOSE BY MOUTH PER KG. BODY WEIGHT*	BEFORE GALACTOSE INTAKE	PERCENTAGE OF BLOOD SUGAR AFTER GALACTOSE INTAKE						GM. SUGAR IN URINE	DIAGNOSIS
						½ H.	1 H.	1½ H.	2 H.	2½ H.	3 H.		
1	F	26	59.0	0.68	0.12	0.17	0.15	0.11	0.11	0.11	0.12	0.20	traumatic neurosis
2	M	17	56.3	0.71	0.11	0.15	0.19	0.14	0.11	0.11	0.11	2.26	chronic polyarthritis
3	F	34	64.7	0.62	0.11	0.16	0.16	0.12	0.10	0.12	0.10	0.85	stomach catarrh (cured)
4	M	31	63.0	0.63	0.11	0.17	0.15	0.11	0.09	0.09	0.09	0.46	pericarditis (cured)
5	F	56	50.0	0.80	0.11	0.12	0.16	0.12	0.10	0.09	0.09	0.30	normal
6	F	39	55.0	0.73	0.12	0.16	0.17	0.12	0.11	0.11	0.11	1.20	pulmonary t. b. (inactive)
7	F	39	65.0	0.62	0.11	0.16	0.13	0.11				0.70	hysteria
Average		35	59.0	0.68	0.11	0.16	0.16	0.12	0.10	0.10	0.10	0.85	

*Forty grams of galactose given to each subject.

alimentary value. The blood-sugar determinations were made by means of Bang's method.

Five patients with outspoken symptoms of tetany were studied. All of these individuals exhibited Trousseau's sign and Chvostek's phenomenon, and their electrical reactions were of the Erb type. The data with reference to these subjects are given in Table I.

Seven individuals who were either normal or had conditions that could have no possible effect upon the galactose tolerance were utilized as controls. The data with reference to the controls are given in Table II.

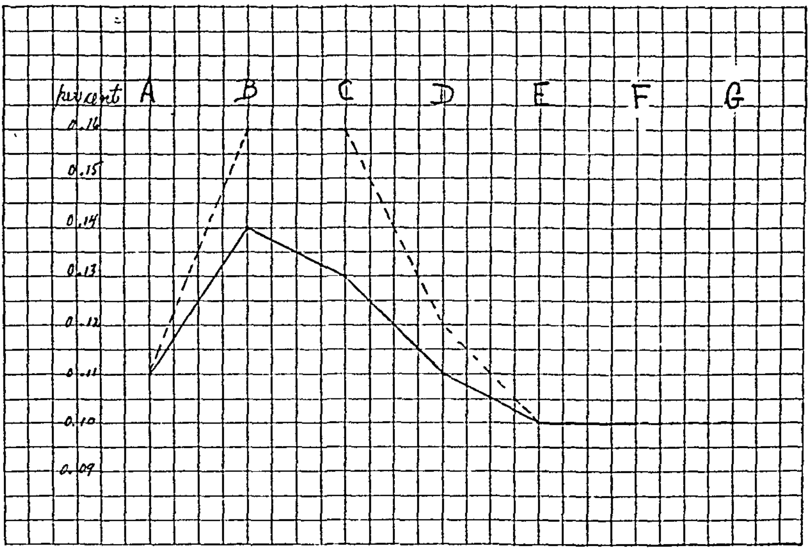

Fig. 1.—Blood-sugar curves after ingestion of approximately 40 gm. of galactose. Solid line, composite curve of five cases of outspoken tetany; interrupted line, composite curve of seven control cases. *A.* before galactose intake; *B.* one-half hour after; *C.* one hour after; *D.* one and a half hours after; *E.* two hours after; *F.* two and a half hours after; *G.* three hours after. Sugar determinations by Bang's method.

The results of this study are shown in Tables I and II and are graphically represented in the accompanying figure. Both the tables and the composite graph (Fig. 1) show that in the 5 cases of human tetany studied there was no decrease in the galactose tolerance; on the contrary, the tolerance was somewhat increased. Thus the changes in the carbohydrate metabolism observed in parathyroidectomized dogs cannot be duplicated in human tetany, and the study of the galactose tolerance does not offer a means of recognizing latent tetany.

SUMMARY

Five patients with outspoken symptoms of tetany and 7 control subjects were studied by means of blood-sugar curves as to their sugar tolerance after the ingestion of approximately 40 gm. of galactose. It was thought that the constant changes in the carbohydrate metabolism observed in parathyroidectomized dogs might possibly be duplicated in human cases of tetany. No decrease, however, in the galactose tolerance was found in these cases; on the contrary, the tolerance to this sugar was relatively somewhat increased. The

study of the galactose tolerance, therefore, does not offer a means of recognizing latent tetany in man.

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THE INFLUENCE OF L. ACIDOPHILUS ON THE COLON-AEROGENES GROUP IN THE INTESTINE*

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THE transformation or simplification of the human intestinal flora by means of L. acidophilus has been considered one of its salient features. In fact this phenomenon has been generally regarded as responsible for the advantages of L. acidophilus therapy. A review of the literature[1] leads one to the conclusion that in the successful treatment of constipation and diarrhea by L. acidophilus there is a relative increase in the number of aciduric organisms, compared with the number of proteolytic bacteria. The natural presumption is that such an increase is actual as well as relative. Yet there has been no experimental data offered in substantiation. The reduction in actual numbers of the colon-aerogenes group in the intestine is of importance not only in evaluating L. acidophilus therapy but in understanding its nature. The present investigation, therefore, was undertaken to throw some light on this problem.

A series of 36 epileptic male patients in the Manhattan State Hospital were subdivided into 3 groups for observation and treatment. Careful daily records were kept with regard to the number of convulsions, as well as the number of defecations, noting amount, color and consistency. These epileptics received the routine hospital diet. No selection of patients for the 3 groups was made other than to have an approximately equal distribution of young and old epileptics. For one month prior to any treatment, during treatment, and for two months subsequently, daily records were kept and weekly fecal analyses made. The methods used have been described elsewhere.[1] In addition to the microscopic examination of gram-stained smears made by our

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modified method[1] and plated on whey agar, a special synthetic medium for the enumeration of organisms of the colon-aerogenes group[2] was employed in the two highest dilutions. A count of actual numbers was made by calculation of samples on the basis of dry weight.

Before considering the influence of L. acidophilus treatment it is of interest to discuss the results of feeding kaolin to epileptics. After the one month of preliminary observation 12 epileptics received 60 gm. of kaolin daily for about two months, 120 gm. of kaolin for the next ten days, and 240 gm. of kaolin for the final three weeks. Bacteriologists have always sought for the etiologic factor in epilepsy in the intestinal tract. If, as has been assumed, toxins are liberated in the intestinal tract of epileptics, then it seemed likely that kaolin, which has great absorbing capacity, would be of value. In a thought-provoking paper by Braafladt[3] it was claimed that kaolin "combines with toxins and toxic products of Vibro cholerae, B. dysenteriae (Shiga), B. enteritidis, B. diphtheriae, B. botulinus, B. typhosus, B. paratyphosus B, and perhaps of putrefactive and proteolytic bacteria, rendering them harmless. When taken by mouth over a period of from ten to thirty days in sufficient quantities (1 to 2 ounces per day for man), it changes the intestinal flora of rats, dogs, and men from a predominantly proteolytic type to an aciduric one."

According to Bergeim,[4] however, kaolin at first decreases and then increases intestinal reduction.

Briefly stated, kaolin had little if any influence in reducing the number of convulsions. Six of the 13 patients had more convulsions during treatments, 4 had approximately the same number, while 2 showed some diminution. The latter were within experimental error as shown by subsequent observations.

Kaolin did have a tendency to increase the number of normal daily defecations in 8 of the 12 patients. This was to be expected because of the improvement in physical character of the feces, which also became lighter in color. It is important, however, to note that there was no transformation from proteolytic to aciduric flora as was anticipated. The relative percentages of this group as calculated in gram-stained smears remained practically constant. On whey agar plates, with two exceptions, no L. acidophilus colonies were found; nor could it be concluded from the colon-aerogenes medium that a reduction in this group occurred.

There were 24 epileptics scheduled to receive L. acidophilus milk; these were divided into two groups. The first group comprised 12 patients, who, after a preliminary month of observation, received 1000 c.c. of sterile milk daily for more than a month. This served as a control for the subsequent ingestion of 1000 c.c. of L. acidophilus milk for a period of two months. Lactose was administered for the first two weeks of the period of L. acidophilus treatment to facilitate a transformation of the intestinal flora. The second group comprised 12 patients, who, after a preliminary month of observation, received 1000 c.c. of L. acidophilus milk for eleven days. Treatment was discontinued because of laboratory difficulties on all but 3 of these patients who were transferred to the first group later.

Considering the first group, the administration of 1000 c.c. sterile milk daily can be said to have had little if any influence on either the number of convulsions or the number of normal defecations. As was to be expected,[1] an increase in the number of normal defecations occurred when the same patients received 1000 c.c. of L. acidophilus milk daily. The physical character of the feces was also improved in the usual way. There was a striking reduction in the number of convulsions in 6 epileptics and a slight reduction in 3 others. These points will be discussed in detail in another communication.[5]

The microscopic examination of the gram-stained fecal smears showed an occasional preponderance of aciduric over proteolytic types. More significant are the results of actual numbers of L. acidophilus colonies appearing on whey agar plates. The percentage of L. acidophilus based on the total number of colonies appearing on the whey plates is shown in Table I. It will be remembered that in this investigation the calculations have been made on the dry basis, the 10 gm. fecal specimens having been air-dried.

Table I

Actual Number of L. Acidophilus Recovered During Treatment (Per Cent)

Case No.	7/15	7/22	7/29	8/5	8/12	8/19	8/26	9/2	9/9	9/16	Average
1	21	66	30	52	37	12				25	42
2			12			63			8		28
3		22	19	5					16	83	29
4	57			6		40		6	30		28
5					65		10	32	56		41
6			50	30				40	100		55
7	42	9					30		10		23
8	100	12	7	55		33	23	33	20	8	32
9	73		53	34	10			86	4	5	38
10	57	80	60	60							64
11			24	24	50		34		40	50	37
12	84	49	48	48	22		31	39	15	47	42
13			40	91	13			85			57
14	24				50						37
15			13					50	92	12	42
16	92				45		15	8	16	40	36

From the data in Table I it will be seen that L. acidophilus appeared on the whey agar plates in varying amount. Only positive findings have here been recorded. At first glance it may seem that a comparatively low recovery of L. acidophilus was obtained. Since the patients received lactose for only two weeks, however, this is not surprising. Viable L. acidophilus have been recovered during treatment where none were previously found on plates of the highest dilutions. The causal relationship between viable L. acidophilus in the intestinal tract and an increase in normal defecations is thus again demonstrated.[1]

What influence have these viable L. acidophilus organisms on the colon-aerogenes group in the human intestine? This is the question of immediate concern. The difficulties in answering it satisfactorily are inherent in the problem. Unquestionably, the most important single factor is the composition and amount of food and fluid intake. In order to establish with any

degree of accuracy the normal number of colon-aerogenes organisms present in any individual, an extremely simple and rigorously controlled diet is essential. Even then considerable variation in numbers might be expected. Such variation was striking in the epileptics under consideration who were receiving the routine hospital diet. It was impossible to control their diet either in amount or composition because of the exigencies of ward management. The results in enumerating the colon-aerogenes organisms in these patients were, therefore, extremely variable.

It would scarcely be of value to publish the tables containing the data obtained. A typical instance alone will suffice. This is recorded in Table II and may be considered representative of the entire series of epileptics studied.

TABLE II

ACTUAL NUMBERS OF COLONIES ON WHEY AGAR AND COLON-AEROGENES MEDIUM FROM FECAL SPECIMENS OF A REPRESENTATIVE EPILEPTIC

(In Millions per Gram Dry Weight)

TREATMENT	DATE	WHEY	L. ACIDOPHILUS	COLI
Nothing	4/21	245.16		60.00
"	4/29	664.89		265.95
"	5/27	240.70		111.60
1000 c.c. Sterile Milk Daily	6/3	2141.38		1070.68
" " " " "	6/24	1436.80		104.10
" " " " "	7/1	3723.90		797.87
" " " " "	7/8	625.00		10.71
1000 c.c. L. Acidophilus Milk and 300 gm. Lactose Daily	7/15	250.00	53.57	8.93
1000 c.c. L. Acidophilus Milk and 300 gm. Lactose Daily	7/22	1428.50	952.30	104.76
1000 c.c. L. Acidophilus Daily	7/29	2074.70	1037.35	95.43
" " " " "	8/5	1623.90	1346.10	85.47
" " " " "	8/12	1351.35	506.75	202.70
" " " " "	8/19	376.47	47.06	2.35
" " " " "	8/26	1320.75		105.66
" " " " "	9/2	961.53		60.43
" " " " "	9/9	491.40		3.68
" " " " "	9/16	1434.85	359.71	59.31
Nothing	9/23	440.93		22.22
"	9/30	1202.68		23.38
"	10/14	174.53		113.21
"	10/21	186.05		158.91
"	10/28	192.11		36.94
"	11/4	138.32		78.68
"	11/18	514.52		107.90

Averages of the actual numbers of the colon-aerogenes group found in individuals before, during, and after treatment have been compiled and are presented in Table III.

Generally speaking, it will be seen from Cases 1 through 12 in Table III that there is an increased number of colon-aerogenes group present when 1000 c.c. of sterile milk are ingested daily as compared with the period before administration. On the other hand, the administration of 1000 c.c. of L. acidophilus milk is responsible for a diminution in the numbers of the colon-aerogenes group when compared with the period during which the same amount of sterile milk was administered. When the period before treatment is compared with the period during which L. acidophilus was administered,

it will be seen that there are 2 cases showing a decrease and 7 cases showing an increase in the colon-aerogenes group. Comparing the period after treatment with that of L. acidophilus administration, it will be noted that there were almost as many cases showing an increase as there were cases showing a diminution in the colon-aerogenes group. The same is true in a comparison of numbers before and after treatment.

Considering the results of the second group of patients, Cases 13 through 24 who received 1000 c.c. L. acidophilus milk and 300 gm. lactose daily for eleven days, it will be seen from Table III that there were more instances of an increase than a decrease in the numbers of the colon-aerogenes group.

TABLE III

AVERAGE NUMBER OF COLON-AEROGENES GROUP BEFORE, DURING, AND AFTER TREATMENT (Expressed in Millions per Gram on Dry Basis)

CASE NO.	BEFORE TREATMENT	DURING 1000 C.C. STERILE MILK DAILY	DURING 1000 C.C. L. ACIDOPHILUS MILK DAILY	AFTER TREATMENT
1	148	496	73	78
2	4	610	18	246
3	55	4191	166	26
4	22	407	61	216
5	69	601	36	39
6	2	34	82	65
7	18	1201	81	4
8	15	24	344	21
9	193	466	17	6
10	75	803	0	–
11	38	332	78	113
12	80	58	57	85
13	38		19	88
14	1827		1699	1780
15	426		435	87
16	42		11	102
17	249		1	255
18	9		1	235
19	17		72	44
20	69		2956	4736
21	5		7	102
22	7		2174	935
23	–		6	121
24	17		38	319

There were, however, more instances of an increase in these organisms after treatment was stopped than there was diminution. With one exception there were greater numbers of the colon-aerogenes group after than there were before treatment.

Averages compiled for the 12 patients receiving kaolin showed that in 9 cases there was an increase in the numbers of the colon-aerogenes group and that in 4 cases there was a diminution. Little importance need, therefore, be attached to kaolin as influencing the bacterial flora in these patients.

From the data presented it would be difficult to arrive at any valid conclusion concerning the influence of L. acidophilus on the colon-aerogenes group in the human intestine. Perhaps the most striking feature is that the ingestion of 1000 c.c. of sterile milk causes an increase in the numbers of these organisms. But a comparison of the period when L. acidophilus was administered with the period before and after treatment leaves much to be desired.

There is evidence that L. acidophilus does diminish the actual number of the colon-aerogenes group, but there is also evidence to the contrary. Undoubtedly, the large experimental error is chiefly responsible for the unsatisfactory nature of these results. The present report, therefore, which briefly outlines a mass of data, is perhaps suggestive rather than conclusive.

SUMMARY

1. Weekly quantitative determinations of bacterial groups in fecal specimens were calculated upon the dry basis in a series of 37 epileptics over a period of five months. Selective media for the enumeration of the colon-aerogenes group and L. acidophilus were employed.

2. Twèlve patients were fed 1000 c.c. L. acidophilus milk daily for two months after receiving 1000 c.c. of sterile milk for a month. Twelve patients received 1000 c.c. of L. acidophilus milk and 300 gm. of lactose daily. Thirteen patients received 60 gm. kaolin daily for two months and an additional amount up to 240 gm. daily for one month. Daily records were kept of convulsions and defecations. For one month before, and for two months after treatment, as well as during treatment, observations and fecal analyses were carried out.

3. L. acidophilus was recovered in varying amount from all patients ingesting L. acidophilus milk for two months. No L. acidophilus was recovered from patients receiving kaolin.

4. Averages of the numbers of the colon-aerogenes group indicated an increase when sterile milk was administered. Compared with this, L. acidophilus caused a diminution. When, however, the period during which L. acidophilus was administered was compared with the periods before and after treatment, diminution in numbers of the colon-aerogenes group occurred in some instances and an increase in others. Kaolin gave similar but more unfavorable results.

5. Then experimental error in this investigation was so great as to preclude clear-cut conclusions.

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STUDIES ON THE AUTONOMIC SYSTEM, III. THE REACTION OF THE CIRCULAR MUSCLE OF THE EXCISED SURVIVING SMALL INTESTINE OF THE FROG (RANA PIPIENS) TO EPINEPHRIN, *PILOCARPIN, ATROPINE, AND BARIUM.**

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INTRODUCTION

THE reaction of the excised surviving longitudinal muscle of the small intestine of the frog (Rana pipiens) to epinephrin, pilocarpin, atropine, and barium was previously reported[1] to be at variance with the results obtained in mammals as reported by previous investigators.

In the following report the effect of the above-mentioned poisons on the circular muscle of the excised surviving small intestine of the frog (Rana pipiens) will be considered.

METHOD OF STUDY

In order to investigate the effect of the above poisons on the circular muscle of the small intestine of the frog, rings measuring from two to three millimeters in length were taken from pithed frogs, about a half inch above the rectum and from a portion of the intestine situated at a similar distance below the stomach. For the sake of comparison, in many instances a longitudinal segment measuring about 10 to 15 mm. was taken from the region immediately below the upper circular ring or above the lower circular ring and mounted with the rings. Both circular rings and the longitudinal segment were then arranged in the usual manner in oxygenated Locke-Ringers solution so as to obtain permanent graphic records. The rings were suspended intact, similar to rubber bands and not as strips. Further details as to the method may be found in the first paper of this series. (See Footnote 1.)

Table I

Saline Solutions

Locke-Ringers Solution	NaCl per cent	KCl per cent	$CaCl_2$ anhydrous per cent	$NaHCO_3$ per cent	NaH_2PO_4 per cent
I	0.7	0.02	0.02	–	–
II	0.8	0.02	0.02	–	–
III	0.7	0.02	0.02	0.05	–
IV	0.7	0.02	0.02	0.1	0.005

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[1]Roth, George B. "Studies on the autonomic system, I. The antagonism of the stimulant action of barium chloride on the excised surviving small intestine of the frog (Rana pipiens) by means of epinephrin, pilocarpin and atropine. Arch. internat. de pharmacod. et de therap., 1923, xxvii, 323.

Experiments were made with several saline solutions; their composition will be found in Table I. It was found that the alkaline solutions were best suited for the development of rhythm; therefore they were used in the majority of the experiments.

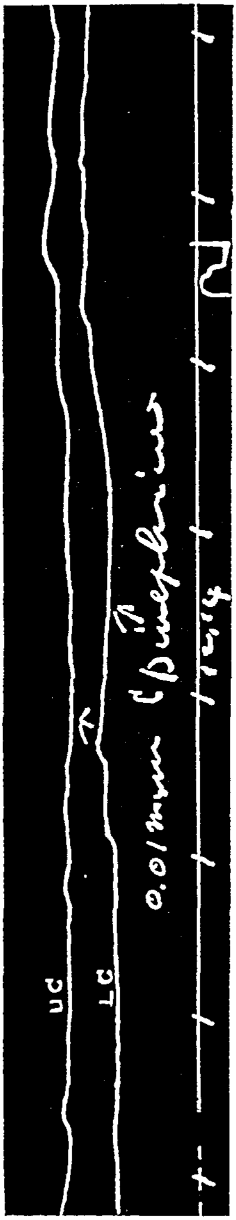

Fig. 1.—(R83D1) Effect of a small dose of epinephrin on the circular muscle of the small intestine of R. pipiens. Oxygenated Locke-Ringers solution No. 1 at 23° C. was employed as the saline solution.

RESULTS OBTAINED

The results obtained in this investigation were not as clear cut as those obtained previously on the longitudinal muscle; perhaps in the present study this was caused by using a saline which permitted rhythm. It was exceedingly

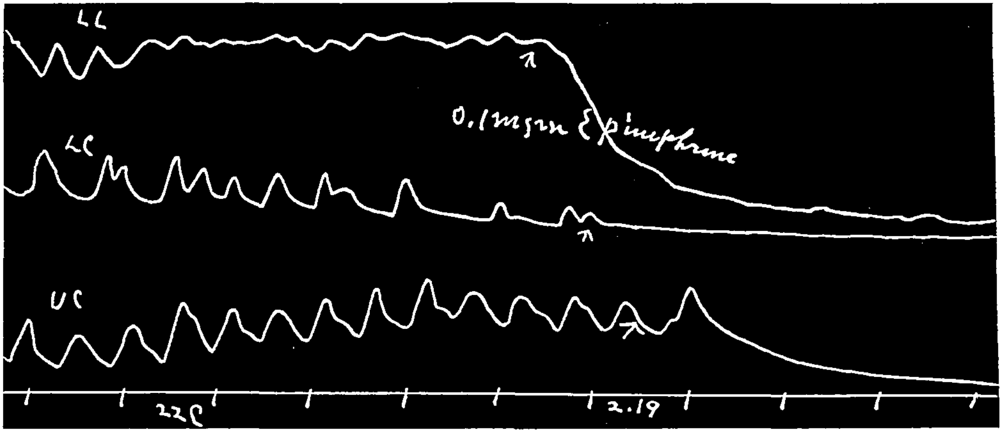

Fig. 2.—(R118D3) Effect obtained from the use of a relatively high concentration of epinephrin on the longitudinal (*LL*) and circular (*LC* and *UC*) muscles of the excised small intestine of R. pipiens. Oxygenated Locke-Ringers No. 3 at 22° C. was used as the saline solution.

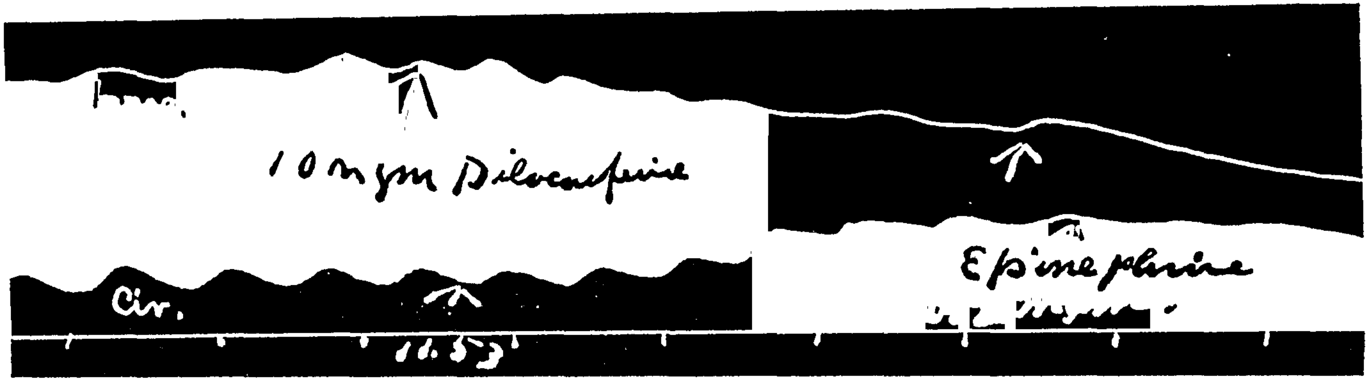

Fig. 3.—(R76D1) Response of longitudinal (*Long.*) and circular (*Cir.*) muscles of the excised small intestine of R. pipiens to 10 mg. of pilocarpin, oxygenated Locke-Ringers solution No. 1at 22° C. being used as the saline solution.

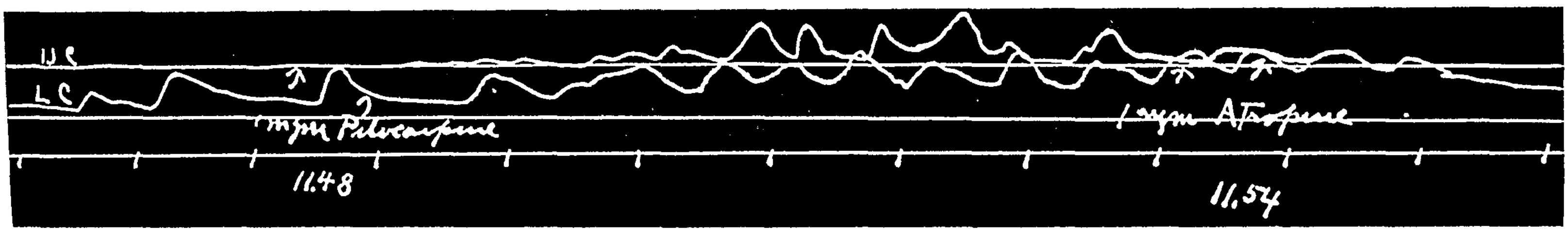

Fig. 4.—(R98D2) Antagonistic action of 1 mg. of atropine against 1 mg. of pilocarpin on the circular muscle of the excised small intestine of R. pipiens when suspended in oxygenated Locke-Ringers solution No. 4 at 19° C.

difficult, therefore, in some cases to rule out spontaneous changes in rhythm, in tonus, or in the amplitude of contraction.

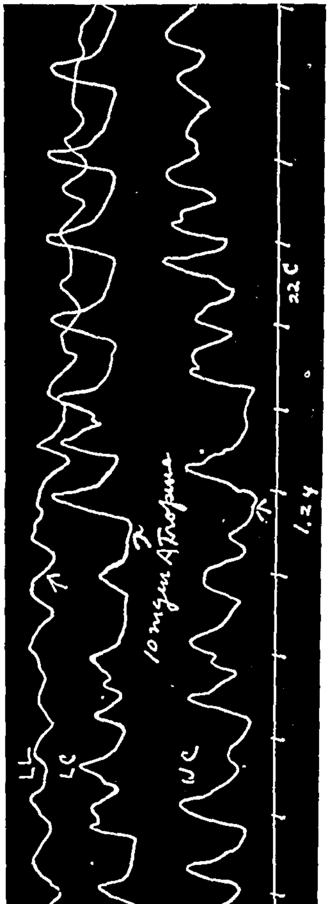

Fig. 5.—(R118D2) Response of longitudinal (*LL*) and circular muscles (*LC* and *UC*) of the excised small intestine of R. pipiens to 10 mg. of atropine when suspended in oxygenated Locke-Ringers solution No. 3 at 22° C. Tonus decreases in the longitudinal (*LL*) and increases in the circular (*LC* and *UC*) preparations.

EPINEPHRIN

The effects obtained from epinephrin depended somewhat upon dosage; that is, 0.01 mg. may increase tonus and the height of rhythmic contraction

or may cause rhythm to appear in a quiescent ring. In other instances 0.01 mg. may depress tonus and abolish rhythm.[2]

In concentrations greater than 0.01 mg. but not exceeding 1 mg., a decrease in tonus with abolition of rhythm usually occurred. Out of 37 experiments in which epinephrin was used, negative results were obtained in 9 instances. (See Figs. 1 and 2.)

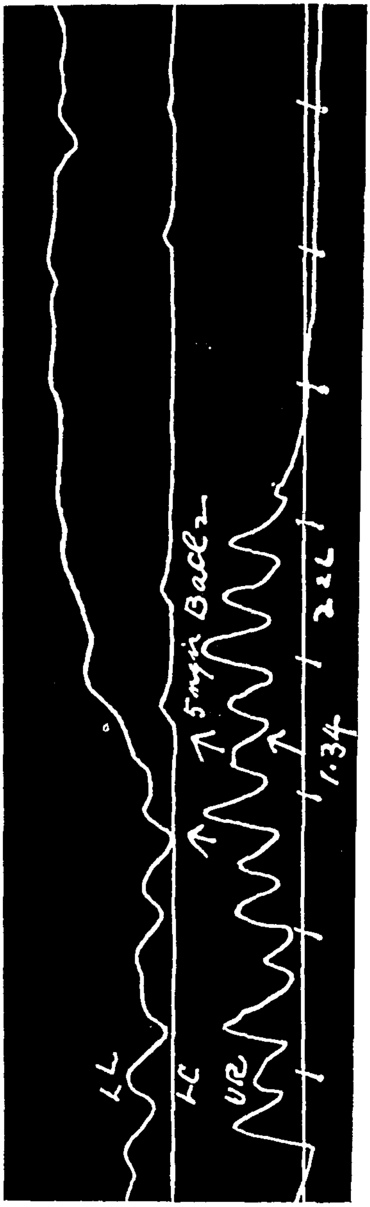

Fig. 6.—(R122D2) Effect of 5 mg. of barium chloride on the longitudinal (*LL*) and circular (*LC* and *UC*) muscles of the excised small intestine of R. pipiens when suspended in Locke-Ringers solution No. 3 at 22° C. Tonus increases in longitudinal, rhythm appears in lower circular, while tonus decreases and rhythm ceases in upper circular preparation.

[2]The quantity of drug referred to in these experiments indicates the amount added to 100 c.c. of the saline solution; for example, when 0.01 mg. was employed, the concentration bathing the tissues was therefore a 1 to 100,000 solution.

PILOCARPIN

In dosages ranging from 0.1 mg. to 10 mg., pilocarpin was found either to produce an increase in tonus, in amplitude, and occasionally in rhythm in the circular muscle of the small intestine of the frog, or to have no effect. (See Figs. 3 and 4.)

As a rule the longitudinal segments which were employed in this investigation for comparison reacted in the same manner to that previously reported, namely, to produce depression. (See Fig. 3.) In 4 out of 33 experiments, however, in which 1 mg. was used, a slight increase in tonus occurred in the longitudinal segment. An increase in tonus likewise occurred in one experiment, after 0.1 and 10 mg. dosages. Apparently these results are due to the fact that in the present investigation the saline employed permitted response to weak stimulating influences.

ATROPINE

Atropine in dosages ranging from 0.1 to 20 mg. produced variable results on the circular muscle of the small intestine of the frog. Usually no response was obtained, but in a few experiments an increase in tonus, amplitude and rhythm occurred (see Fig. 5), while in other experiments a slight decrease in tonus, amplitude, and rhythm was produced. Atropine in equal dosage was able to completely antagonize the stimulating action of pilocarpin. (See Fig. 4.)

BARIUM

The experiments with barium chloride on the circular muscle of the excised small intestine of the frog yielded results similar to those with atropine, namely, no response or in a few experiments either stimulation or depression was obtained. The dosages employed ranged from 0.1 mg. to 20 mg. The results were independent of dosage. In Fig. 6, for example, 5 mg. caused rhythm to appear in the lower circular ring, while in the upper one tonus was markedly depressed and rhythm disappeared.

SUMMARY

The response of the circular muscle of the excised surviving small intestine of the frog (Rana pipiens) to epinephrin, pilocarpin, atropine, and barium is essentially the same as that of the longitudinal muscle; the principal difference is that pilocarpin is primarily a stimulant to the circular ring, whereas it is mainly a depressant to the longitudinal muscle. This phenomenon is believed to be unique. Epinephrin in small doses may act as a stimulant or depressant to tonus, rhythm, and amplitude of contraction, while more concentrated solutions are usually depressant. Pilocarpin is primarily a stimulant. Atropine is usually ineffective but may be either stimulant or depressant. It is able to antagonize pilocarpin completely in equal dosage. Barium, like atropine, usually yielded negative results but in a few experiments either stimulation or depression was obtained. The results were independent of dosage.

A standard diet was prepared as follows for a 15 kg. dog:

Fresh meats	–	250 gm.
Dog Biscuits	–	60 gm.
Lard	–	30 gm.
Bone-ash	–	10 gm.
		350 gm.

This formula was prepared in large quantities as follows: the biscuits and meat were ground with a meat grinder, the lard and bone-ash added, mixed thoroughly, and finally the whole mass was run a second time through the grinder. For each quantity prepared, the nitrogen content was determined.

The dogs were weighed, placed in metabolism cages, fed once a day (in the morning), urine collected every twenty-four hours (in the morning), and the percentage of uric acid determined by the Hopkins Method, Folin's modification, Simon, *Clinical Diagnosis,* p. 378. The drugs were given when the dogs maintained a constant uric acid excretion.

C. Protocols.—Following are the results of the experiments:

Dog 1

Female—Wt. = 12.6 Kg.

DATE	DRUG	AMOUNT OF URINE	URIC ACID PERCENTAGE	DIET	NITROGEN PERCENTAGE
6– 4		675 c.c.	0.360	300 gm.	3.30
6– 5		520 c.c.	0.388	" "	"
6– 6		275 c.c.	0.380	" "	"
6– 7		475 c.c.	0.310	" "	"
6– 8		465 c.c.	0.312	" "	"
6– 9		425 c.c.	0.336	" "	"
6–10	Phenylcinchoninic	280 c.c.	0.337	" "	"
6–11	Hydrochlorid	515 c.c.	0.620	" "	"
6–12	(Chloroxyl) 1 gm.	320 c.c.	0.420	" "	"
6–13		290 c.c.	0.360	" "	"

DATE	DRUG	AMOUNT OF URINE	URIC ACID PERCENTAGE	DIET	NITROGEN PERCENTAGE
6–17		390 c.c.	0.357	300 gm.	2.83
6–18		400 c.c.	0.355	" "	"
6–19	Phenylcinchoninic	185 c.c.	0.365	" "	"
6–20	Hydriodid	610 c.c.	0.523	" "	"
6–21	(Oxyliodid) 1 gm.	270 c.c.	0.280	" "	"
6–22		450 c.c.	0.323	" "	"
6–23		365 c.c.	0.368	" "	"
6–24	Phenylcinchoninic	325 c.c.	0.300	" "	"
6–25	acid 1 gm.	420 c.c.	0.412	" "	"
6–26		235 c.c.	0.356	" "	"
6–27		225 c.c.	0.193	" "	"

DATE	DRUG	AMOUNT OF URINE	URIC ACID PERCENTAGE	DIET	NITROGEN PERCENTAGE
10–21		250 c.c.	0.524	300 gm.	3.72
10–22		500 c.c.	0.526	" "	"
10–23		190 c.c.	0.469	" "	"
10–24		330 c.c.	0.471	" "	"
10–25		140 c.c.	0.549	" "	"
10–26		485 c.c.	0.551	" "	"
10–27		200 c.c.	0.516	" "	"
10–28		375 c.c.	0.514	" "	"

DATE	DRUG	AMOUNT OF URINE	URIC ACID PERCENTAGE	DIET	NITROGEN PERCENTAGE
10-29		225 c.c.	0.400	300 gm.	3.72
10-30	Phenylcinchoninic	160 c.c.	0.430	" "	"
10-31	Hydrochlorid	450 c.c.	0.853	" "	"
11- 1	(Chloroxyl) 1 gm.	250 c.c.	0.620	" "	"
11- 2		250 c.c.	0.450	" "	"
11- 3		220 c.c.	0.480	" "	"
11- 4		250 c.c.	0.480	" "	"
11- 5	Phenylcinchoninic	260 c.c.	0.400	" "	"
11- 6	Hydriodid	360 c.c.	0.610	" "	"
11- 7	(Oxyliodid) 1 gm.	230 c.c.	0.450	" "	"
11- 8		300 c.c.	0.480	" "	"

Dog 2
Female—Wt. = 15 Kg.

DATE	DRUG	AMOUNT OF URINE	URIC ACID PERCENTAGE	DIET	NITROGEN PERCENTAGE
6-14		530 c.c.	0.292	350 gm.	2.83
6-15		515 c.c.	0.288	" "	"
6-16		575 c.c.	0.300	" "	"
6-17		525 c.c.	0.280	" "	"
6-18		515 c.c.	0.235	" "	"
6-19	Phenylcinchoninic	475 c.c.	0.233	" "	"
6-20	Hydriodid	510 c.c.	0.460	" "	"
6-21	(Oxyliodid) 1 gm.	650 c.c.	0.471	" "	"
6-22		500 c.c.	0.367	" "	"
6-23		470 c.c.	0.283	" "	"

DATE	DRUG	AMOUNT OF URINE	URIC ACID PERCENTAGE	DIET	NITROGEN PERCENTAGE
12- 6		525 c.c.	0.360	350 gm.	3.67
12- 7		415 c.c.	0.370	" "	"
12- 8		790 c.c.	0.350	" "	"
12- 9		275 c.c.	0.390	" "	"
12-10		335 c.c.	0.340	" "	"
12-11	Phenylcinchoninic	390 c.c.	0.390	" "	"
12-12	Hydrochlorid	610 c.c.	0.620	" "	"
12-13	(Chloroxyl) 1 gm.	360 c.c.	0.320	" "	"
12-14		440 c.c.	0.430	" "	"
12-15		370 c.c.	0.390	" "	"
12-16	Phenylcinchoninic	475 c.c.	0.390	" "	"
12-17	Hydriodid	310 c.c.	0.430	" "	"
12-18	(Oxyliodid) 1 gm.	380 c.c.	0.380	" "	"
12-19		500 c.c.	0.430	" "	"
12-20	Quinine Phenyl-	415 c.c.	0.430	" "	"
12-21	cinchoninate	650 c.c.	0.570	" "	"
12-22	(Quinoxyl) 1 gm.	360 c.c.	0.370	" "	"
12-23		525 c.c.	0.370	" "	"
12-24		435 c.c.	0.370	" "	"
12-25		315 c.c.	0.370	" "	"
12-26	Phenylcinchoninic	400 c.c.	0.370	" "	"
12-27	acid 1 gm.	650 c.c.	0.560	" "	"
12-28		450 c.c.	0.550	" "	"
12-29		400 c.c.	0.450	" "	"
12-30		425 c.c.	0.380	" "	"

Dog 3
Male—Wt. = 21 Kg.

DATE	DRUG	AMOUNT OF URINE	URIC ACID PERCENTAGE	DIET	NITROGEN PERCENTAGE
10-25		315 c.c.	0.510	400 gm.	3.72
10-26		485 c.c.	0.570	" "	"
10-27		410 c.c.	0.490	" "	"
10-28		360 c.c.	0.530	" "	"
10-29		340 c.c.	0.550	" "	"
10-30		325 c.c.	0.520	" "	"
10-31		265 c.c.	0.420	" "	"

DATE	DRUG	AMOUNT OF URINE	URIC ACID PERCENTAGE	DIET	NITROGEN PERCENTAGE
11- 1		325 c.c.	0.340	400 gm.	3.72
11- 2	Phenylcinchoninic	225 c.c.	0.270	" "	"
11- 3	Hydrochlorid	350 c.c.	0.560	" "	"
11- 4	(Chloroxyl) 1 gm.	270 c.c.	0.340	" "	"
11- 5		225 c.c.	0.350	" "	"
11- 6		230 c.c.	0.490	" "	"

DATE	DRUG	AMOUNT OF URINE	URIC ACID PERCENTAGE	DIET	NITROGEN PERCENTAGE
11–15		275 c.c.	0.370	400 gm.	3.29
11–16		360 c.c.	0.330	" "	"
11–17		315 c.c.	0.360	" "	"
11–18	Phenylcinchoninic	300 c.c.	0.320	" "	"
11–19	acid 1 gm.	325 c.c.	0.480	" "	"
11–20		970 c.c.	0.460	" "	"
11–21		1300 c.c.	0.160	" "	"
11–22		1075 c.c.	0.200	" "	"
11–23		975 c.c.	0.160	" "	"
11–24		750 c.c.	0.190	" "	"

Dog 1 gave a constant normal uric acid value. Following the oral administration of 1 gm. of phenylcinchoninic hydrochlorid (chloroxyl), the uric acid content increased from a normal average of 0.346 to 0.620 gm. in twenty-four hours and returned to normal in forty-eight hours. One gram of phenylcinchoninic hydriodid (oxyliodid) and 1 gm. of phenylcinchoninic acid, respectively, were given and produced a distinct increase in the uric acid excretion. One gram of phenylcinchoninic hydrochlorid (chloroxyl) and 1 gm. of phenylcinchoninic hydriodid (oxyliodid), respectively, again repeated the same distinct increase in uric acid output.

Dog 2, following a constant normal uric acid value, was given the same drugs, also quinine phenylcinchoninate (quinoxyl). These drugs all produced a similar increase in uric acid excretion.

Dog 3 was given 1 gm. of phenylcinchoninic hydrochlorid (chloroxyl) and phenylcinchoninic acid. Each drug produced a distinct increase in the uric acid content.

III. CONCLUSION

1. The excretion of uric acid of the Dalmatian Coach dog is comparable to that of the human uric acid metabolism.

2. The uric acid eliminants produce a distinct increase in the uric acid excretion.

3. It is possible to determine the value of these drugs as uric acid eliminators by the percentage of uric acid excretion of Dalmatian dogs.

The writer is much indebted to Dr. A. L. Walters and Miss Lucile Carter for their suggestions and criticisms.

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A COMPARISON OF THE CLEVELAND AND THE KOLMER MODIFICATIONS OF THE WASSERMANN TEST*

By Frank R. Lyne, M.D., Cleveland, Ohio

IN 1924 the Cleveland Academy of Medicine appointed a committee, with Dr. H. N. Cole as chairman, to investigate the various modifications of the Wassermann test in common use. This was done with the idea of recommending as a standard system some one method to the laboratories of Cleveland. As a result of the activities of this committee a method was devised which has been called the Cleveland modification.[1, 2] A brief résumé of that method will be given here, emphasizing those points in which it differs from others now in vogue.

The method for preparing antigen was devised by Ecker.[3] Thirty grams of dried beef heart are extracted with 150 c.c. acetone in a reflux condenser over a water-bath for four hours. The mixture is chilled, filtered, and the powder dried. This is now extracted in the same apparatus with 150 c.c. acetone-free, methyl alcohol for the same period of time. The mixture is now filtered while hot and the filtrate evaporated to 90 c.c. For use, the antigen is diluted to one-fourth its anticomplementary unit by adding 0.9 per cent saline solution drop by drop to the proper amount of extract (usually about 1:30). I prefer to reinforce the antigen with 0.2 per cent cholesterol.[1]

Sheep cells are sensitized by adding to 50 c.c. of 5.0 per cent cell suspension, 50 c.c. of a dilution of antisheep hemolysin containing 1000 units; thus each 0.5 c.c. of the mixture contains 5 units. The cell suspension is rotated while adding the hemolysin, and the mixture is allowed to stand in the water-bath at 37° C. for thirty minutes before use. One-half c.c. of the mixture is used in each tube of the complement titration and of the reactions.

Complement is the pooled serum of guinea pigs. It is titrated by placing in a series of tubes the following amounts of 1:30 dilution: 0.09 c.c., 0.12 c.c., 0.15 c.c., etc., to 0.30 c.c. One-half c.c. of sensitized cell suspension is added, then the volume of each tube is brought to 1.5 c.c. with saline solution. The tubes are placed in the water-bath at 37° C. for thirty minutes. The titer is clear-cut and easily read, the unit being the least amount that produces complete hemolysis. Two units, in either 1:10 or 1:15 dilution, are used in the reactions.

Serums to be tested are inactivated at 56° C. for twenty-five minutes. Titration of other reagents used has been given elsewhere.[1]

Reactions are set up as follows: three tubes are arranged for each serum. Tube No. 1 contains 0.1 c.c. serum, tube No. 2 contains 0.05 c.c., and

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No. 3 which is the control contains 0.1 c.c. Tubes Nos. 1 and 2 now receive 0.1 c.c. of freshly prepared antigen dilution; the contents are mixed and allowed to remain at room temperature for fifteen minutes. Two units of complement dilution are added to each tube, and the tubes are placed in the ice box at 6° to 8° C. for fifteen to eighteen hours. Nine-tenths per cent saline solution is then added to each tube to a volume of 1.0 c.c., and 0.5 c.c. sensitized cell suspension is added when the tubes are placed in the water-bath at 37° C. for thirty minutes to permit hemolysis. Readings may be made at once, but preferably after partial sedimentation has taken place.

The Cleveland modification has several advantages. Under proper conditions of preservation the hemolysin does not require titration more often than every six weeks or two months. The complement titrations give clear-cut end points. The small total volume (1.5 c.c.) in both titrations and tests saves reagents and increases the velocity of the reactions. The use of large doses of hemolysin (5 units) renders unnecessary daily titration, increases the velocity of the reactions, especially in the complement titration, and obviates those false negatives due to the presence of native antisheep hemolysis in human serum.[1] In spite of the fact that this introduces a large amount of rabbit serum into the reaction, this is more than compensated by the reduction in the amount of guinea pig serum. Thus the total amount of foreign serum is materially reduced.

Probably the most widely employed modification of the Wassermann test is that of Kolmer. This method is sensitive, specific, and has given satisfactory results in many laboratories. It differs from the Cleveland method in a number of respects, notably in the daily titration of hemolysin, the use of only two units of hemolysin, a weaker cell suspension, and the use of unsensitized cells, and that it requires about twice as much complement for the same number of reactions. The Kolmer technic used in this department is the qualitative method outlined in "Laboratory Diagnostic Methods."[4]

Through the kindness of Dr. Kolmer the writer spent some time in his laboratory studying the method under his supervision, and the antigen used in the comparative tests described herein was provided by him.

The comparison of the Kolmer and the Cleveland modifications was carried out on a series of one thousand consecutive sera received in the laboratory. These were from the Medical Department of St. Alexis Hospital, Dr. Richard Dexter, director, and from the Department of Dermatology of the Cleveland City Hospital, Dr. H. N. Cole, director. Of these sera, 680 were negative in both; 245 gave equally positive reactions. The Kolmer system gave stronger results with 20, while the Cleveland system gave stronger results with 38.

Where one system was positive and the other negative, 3 were positive in the Kolmer system, 2 of these being known syphilitic cases under treatment; 11 were positive in the Cleveland system, 9 being known syphilitic cases. Seventeen sera were anticomplementary in the Kolmer, 15 in the Cleveland system. These results may be tabulated as follows:

Negative in both systems: 680
Equally positive in both: 245
Stronger positive results in the Kolmer system: 20
Stronger positive results in the Cleveland: 38
Positive in the Kolmer, negative in the Cleveland system: 3 (2 known syphilitic)
Positive in the Cleveland, negative in the Kolmer system: 11 (9 known syphilitic)
Anticomplementary in the Kolmer: 17
Anticomplementary in the Cleveland: 15.

It is obvious that there is no significant difference in sensitivity and specificity between the two systems.

In the above study an antigen reinforced with 0.2 per cent cholesterol was used in the Cleveland system. Using an antigen of the same type, but without reinforcement with cholesterol, the following results were obtained:

Negative in both systems: 680
Equally positive in both: 225
Stronger positive results in the Kolmer system: 50
Stronger positive results in the Cleveland: 28
Positive in the Kolmer, negative in the Cleveland system: 5 (4 known syphilitic)
Positive in the Cleveland, negative in the Kolmer: 7 (5 known syphilitic)
Anticomplementary in the Kolmer: 17
Anticomplementary in the Cleveland: 15.

Here again it is apparent that there is no significant variation between the two systems. It should be remembered, however, that the Kolmer antigen contains 0.2 per cent cholesterol.

The Cleveland modification, then, is as sensitive and as specific as the Kolmer. The points of advantage that recommend the former most highly have been mentioned. It offers a decided saving in time, for the time consumed in the titration of reagents each day the test is done is only the thirty minute incubation used in the complement titration. This titer is always clear-cut and the results unmistakable, which often is not the case with the hemolysin and complement titrations using the Kolmer technic with the same reagents. The amount of complement used in the tests is about half that required for the same number of sera in the Kolmer system; this is an important item, especially in smaller laboratories where guinea pigs are bled from the heart. Larger amounts of hemolysin are used, it is true; but this is not of such practical importance, since we are able to preserve this reagent in large amounts over a long period of time. Positive reactions are not obscured by excess native antisheep hemolysin in the patient's serum. A very decided advantage is that the preparation of antigen is readily carried out, requiring only two periods of extraction of four hours each—the product is easily completed in two days. Anticomplementary reactions are encountered in about the same proportion in both systems.

SUMMARY

An analysis of results obtained with 1000 consecutive sera submitted to the Wassermann reaction using both the Kolmer and the Cleveland modifications shows them to be equal in sensitivity and specificity.

The Cleveland modification is uninfluenced by native antisheep hemolysin often present in human serum, is decidedly economical as to time and reagents, and for these reasons should be the method chosen.

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UNION BETWEEN ANTIGEN AND ANTIBODY IN THE WASSERMANN TEST*

By S. L. Leiboff, A.M., New York City

THE question whether the antigen used in the Wassermann test for syphilis unites chemically with the specific substance or substances present in syphilitic serum, or whether there is a mere change in the physical state of the colloids, has never been proved. We know almost nothing about the specific substances present in syphilitic serum. We do know, however, something about the nature of the antigen. Noguchi[1] showed it to be a phosphatid. Porges and Myer[2] believed the particular phosphatid to be lecithin. Sachs and Rondoni[3] used synthetic antigens which contained lecithin as a basis; however, the results obtained were not as good as those obtained with alcoholic liver extracts. Most likely alcoholic liver extracts contain a mixture of various phosphatids. According to MacLean[4] a great number of substances regarded as distinct phosphatids are only mixtures of various lipoids, since it is very difficult to isolate lipoidal substances in a chemically pure form. This difficulty is further complicated by the property of being intersoluble which these bodies possess.

There are four phosphatids as to whose existence as chemical individuals there is definite evidence; viz., lecithin, cephalin, cuorin, and sphingomyelin. They are characterized by containing phosphorus, nitrogen, and fatty acids; they are insoluble in acetone; they form compounds with cadmium chloride by which they are precipitated; they combine with proteins;[4] on hydrolysis they yield phosphoric acid (in the case of the first three phosphatids, in the form of glycerophosphoric acid), various fatty acids, and basic bodies such as cholin:

$$(CH_3)_3{-}N\begin{matrix} \diagup OH \\ \diagdown C_2H_4{-}OH \end{matrix}$$

*From the Biochemical Department of Lebanon Hospital Laboratory, New York City.
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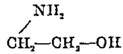
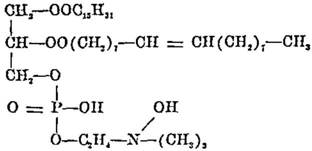
and amino-ethyl alcohol:



The best known phosphatid is lecithin to which the following formula is assigned:



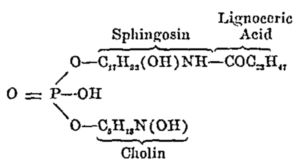
Thus we may regard lecithin as a fat in which one of the three fatty acids is substituted by phosphoric acid combined with the base cholin.

Cephalin resembles lecithin very much; it differs in possessing a different base, amino-ethyl alcohol instead of cholin, and possibly different fatty acids. According to recent workers[4] lecithin contains palmitic and oleic acids, while cephalin contains stearic and linoleic acids. The structure of cuorin is less understood; it resembles lecithin, and, according to Erlandsen[5] contains three fatty acids in the molecule, and a base, probably aminoethyl alcohol. Each of the three phosphatids described contains at least one fatty acid of the unsaturated variety. This makes them exceedingly liable to oxidation changes.

Sphingomyelin contains no unsaturated fatty acids and is, therefore, more stable. Its exact structure is not yet known. Levene[6] prepared what he thought was pure sphingomyelin and obtained the following products on hydrolysis: (1) phosphoric acid (no glycerin); (2) two fatty acids, one probably a hydroxy acid; (3) two bases: cholin and sphingosin. Sphingosin is said to have the following formula:

$$CH_3—(CH_2)_{11}—CH = CH—CH(OH)—CH(OH)—CH_2(NH_2)$$

Levene suggests the following formula for sphingomyelin:



The phosphatids are generally classified according to the method introduced by Thudichum;[7] they are divided into groups depending upon the relation of nitrogen to phosphorus present in the molecule. Thus in lecithin and cephalin one atom of nitrogen and one of phosphorus occur; the N:P ratio is, therefore, 1:1. In sphingomyelin the N:P ratio is 2:1, while in cuorin the N:P ratio is 1:2. Thus lecithin and cephalin are monoaminomonophosphatids; cuorin is a monoaminodiphosphatid; while sphingomyelin is a diaminomonophosphatid.

The alcoholic extracts used as antigens in the Wassermann test consist of mixtures of the various phosphatids in which lecithin predominates by far, since it is the most abundant in the tissue and the most soluble in alcohol.

If the union between antigen and antibody is chemical in nature, then judging from the chemical structure of lecithin there are two ways possible by which a chemical union may take place between it and the positive serum: (1) at the double bonds of the unsaturated fatty acids; or (2) at the free hydroxyl groups present in the phosphoric acid and cholin in the lecithin molecule.

Noguchi's[8] statement that there seems to be a certain relationship between the iodine value and antigenic activity tends to indicate that the chemical union, if any, takes place at the double bond of the fatty acid in the lipoid molecule. To find out whether such is or is not the case, I saturated the double bond with hydrogen by the method of Paal and Oehme.[9] This method consists in shaking with hydrogen an alcoholic solution of lipoidal substance in the presence of a catalyzer, colloidal palladium.

PROCEDURE

Saturation of Double Bonds.—*Preparation of Colloidal Palladium.*—Colloidal palladium was prepared according to the method of Kelber and Schwarz[10] which was somewhat modified. One gram of metallic palladium was heated with a few c.c. of concentrated HCl. The palladium dissolves with great difficulty in HCl, but on adding a few drops of con. HNO_3 it dissolves very readily, and a dark-red solution of palladium chloride is obtained. The free acid was evaporated off and the dry powder washed a few times with water and finally dissolved in 50 c.c. of water. One gram of gelatin was warmed in a small flask with 50 c.c. of water until it dissolved. The palladium chloride solution was made slightly alkaline with dilute ammonia and run into the gelatin slowly, drop by drop with constant stirring. Hydrazine-hydrate (50 per cent Kaulbaum) was then added from a burette drop by drop while stirring constantly. The liquid frothed, giving off gas, and became deep brownish-black. The frothing stopped, and the reduction was completed. The colloidal palladium was then dialyzed in a collodion bag against running water until the outside water gave no precipitate with $AgNO_3$, showing that all the chloride was removed. The palladium was then evaporated down to a small volume on the water-bath and, finally, in a vacuum, resulting in black lustrous scales.

The syphilitic antigen for this experiment was prepared at a low temperature. The muscle tissue of ten beef hearts was ground and spread in thin layers over sheets of cheesecloth stretched on wire frames and dried under the electric fan for thirty-six hours; at the end of this time it was completely dry. It was then ground to a fine powder. Five hundred grams of this powder was placed in a bag made of heavy filter-cloth. The mouth of the bag was supported by a rim of heavy wire which was held by four wires attached to a cork fitted in the wide neck of a twenty liter bottle. The top of the cork was placed a few millimeters below the upper end of the neck

of the bottle and was covered with mercury to make it air-tight. Three reflux condensers were placed in the cork. Two liters of ether were placed in the bottle. The lower end of the cloth-bag was about three inches above the surface of the ether. The bottle was then placed into a large pail of water which was kept warm over a small flame. The extraction with boiling ether was continued for forty-eight hours, at the end of that time the extraction was complete, since a subsequent extraction with fresh ether gave no coloration to the ether, and evaporation of 100 c.c. of the extracted ether left no deposit. The powder was then removed from the bag and dried in an incubator at 37° C. and extracted in the same cloth-bag with a liter of absolute alcohol in the same manner as the ether extraction, except that only one large size reflux condenser was used. This was attached to a vacuum pump and the boiling point of the alcohol thus kept between 35° and 37° C. This extraction was continued for forty-eight hours. The alcoholic extract was then evaporated to dryness in a vacuum for three weeks. A sticky brownish mass was obtained which weighed 46 grams.

Two grams of the dry substance was dissolved in 95 c.c. of alcohol to which was added one c.c. of glacial acetic acid. Two-tenths gram colloidal palladium was dissolved in 5 c.c. of water (it had to be warmed to be brought in solution). The colloidal palladium solution was then placed in a bottle, and the alcoholic lipoidal solution was added to it slowly, when a homogeneous dark suspension was formed. The bottle containing the mixture was then made air-tight and connected by a piece of soft rubber tubing to a reservoir filled with hydrogen under pressure. The hydrogen was prepared from zinc and sulphuric acid in a Kipp's apparatus, and the gas was passed through successive solutions of $KMnO_4$, $AgNO_3$, and KOH into a reservoir under water pressure. Great care had to be taken to remove all traces of arsenic and antimony from the materials used in the preparation of the hydrogen, for they destroy the catalytic action of the palladium. On leaving the reservoir the hydrogen was dried by passing through concentrated H_2SO_4 and anhydrous $CaCl_2$.

The bottle containing the lipoidal solution was then attached to a shaking apparatus connected to an electric motor and shaken for forty-eight hours with the hydrogen under pressure.

The colloidal mixture was then neutralized with ammonia, heated until the palladium coagulated, and filtered. The filtrate was then evaporated to dryness in a vacuum.

One gram of the hydrogenated lipoid was used for an iodine value determination to tell the degree of saturation. The rest was dissolved in 100 c.c. of alcohol and filtered; this was used for the antigenic titration.

A control was prepared by treating two grams of the lipoid in the same way, with the exception of leaving out the hydrogen.

The iodine number was determined by the Hübl method.[11] It showed a distinct reduction in the iodine value in the hydrogenated lipoid:

Iodine number of hydrogenated lipoid	12
Iodine number of nonhydrogenated lipoid	47.

While hydrogenation was not complete, yet enough saturation had taken place to impair seriously the antigenic property of the lipoid if the antigenic property was to depend upon the unsaturated bonds in the lipoidal molecule.

The antigenic titrations were done with an antisheep hemolytic system, one-fourth Wassermann quantities being used. Complement fixation was done in the ice box for four hours; sensitized red cells were then added, and the second incubation was done in the water-bath at 37° C. for twenty minutes and the results read.

The antigens were made up in two dilutions: 1:10 and 1:40 by slowly adding saline from a pipette to a measured amount of antigen into a flask, shaking it all the while.

A series of 13 test tubes were set up; the first 4 tubes receiving increasing amounts of the 1:40 diluted antigen, and the remaining tubes receiving increasing amounts of the 1:10 diluted antigen.

The first 6 tubes contained 0.05 c.c. syphilitic serum each, thus serving as a test for the antigenic power of the antigen. Tubes 7 to 11 inclusive contained no serum (except tube 7 which contained 0.1 c.c. of normal serum and served to test the specificity of the antigen) and demonstrated the anticomplementary property. The last two tubes contained no serum and no complement and showed the hemolytic power of the antigen.

The following signs were used in writing down the results:

++++ = complete inhibition of hemolysis
+++ = slight hemolysis
++ = half hemolysis
+ = slight inhibition
0 = complete hemolysis.

The titration in the following table shows that the binding power of an antigen used in the complement-fixation test for syphilis is not destroyed by saturating the double bonds in the unsaturated fatty acids in the lipoid molecule.

Table I

Titration of Saturated Antigen

ANTIGENIC DILUTION	1-40				1-10								
PROPERTIES	ANTIGENIC						ANTICOMPLEMENTARY					HEMOLYTIC	
Tube	1	2	3	4	5	6	7	8	9	10	11	12	13
Ant. c.c.	.01	.02	.05	0.1	.05	0.1	0.2	0.5	.75	1.0	1.2	0.5	1.0
Saturated Ant.	0	0	0	++	++++	++++	0	0	0	+	++	++++	+++
Control	0	0	0	+++	++++	++++	0	0	0	++	+++	++++	++++

SUBSTITUTION OF HYDROXYL GROUPS

The next step was to find out if the OH groups in the lipoids were responsible for the binding power of the antigen. The most convenient method to accomplish this is to block off the OH groups by introducing acetyl radicles in their place. This may be done by treating a lipoid with acetic anhydrid. The following reaction takes place:

$$R{-}OH + \begin{array}{l} CH_3{-}CO \\ \qquad\qquad \diagdown \\ \qquad\qquad\quad O \\ \qquad\qquad \diagup \\ CH_3{-}CO \end{array} = R{-}OOC{-}CH_3 + CH_3{-}COOH$$

PROCEDURE

Four grams of the lipoidal substance prepared in the previous experiment were heated for two hours under a reflux condenser with 10 c.c. of freshly distilled acetic anhydrid. The acetylated lipoid was then emulsified with about 30 c.c. of water and shaken up with an excess of acetone in which the lipoid precipitated. The precipitate was then washed with fresh portions of acetone a few times. It was then emulsified with about 30 c.c. of water and dialyzed in a parchment bag against running water for forty-eight hours, thus removing all the acetic acid and the anhydrid. The lipoidal material was then precipitated with acetone and dried in a vacuum for two weeks.

One gram of the dry substance was dissolved in 50 c.c. of alcohol and titrated for antigenic properties as in the previous experiment.

As a control four grams of the lipoidal substance prepared in the previous experiment were treated similarly except that the acetylation was left out. One gram of the dry substance was dissolved in 50 c.c. of alcohol.

As the titration in the table below shows, the substitution of CH_3COO groups for the OH groups in the lipoidal molecules does not destroy their antigenic properties.

TABLE II

ACETYLATION OF LIPOID

ANTIGENIC DILUTION	1-40			1-10									
PROPERTIES	ANTIGENIC						ANTICOMPLEMENTARY					HEMOLYTIC	
Tube	1	2	3	4	5	6	7	8	9	10	11	12	13
Ant. c.c.	.01	0.2	.05	0.1	.05	0.1	0.2	0.5	.75	1.0	1.2	0.5	1.0
Acetylated Ant.	0	0	0	+	++++	++++	0	0	+	++	++++	++++	++++
Control	0	0	0	+++	++++	++++	0	0	0	+	++++	++++	++++

SUMMARY

1. Saturation of the double bonds in the fatty acids of the lipoidal antigen used for complement-fixation test in the diagnosis of syphilis, does not materially interfere with its antigenic properties; hence the unsaturated fatty acids in the lipoid molecule are not responsible for the specificity of the Wassermann reaction.

2. Blocking off the hydroxyl groups in the lipoid molecule by substituting acetyl groups does not materially impair the antigenic value of the lipoid.

Since chemical union would most likely take place either at the double bonds of the unsaturated fatty acids or at the hydroxyl groups, and the blocking of both of these possibilities does not materially diminish the specific value of the antigen, it follows that the Wassermann reaction is probably not based upon a chemical reaction, but rather that physical phenomena are involved in it.

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THE SIGNIFICANCE OF BLOOD CULTURES*

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AS IT was thought that the large clinical and pathologic facilities at Bellevue Hospital would afford opportunities for studying the general value of taking blood cultures from the diagnostic, therapeutic and prognostic standpoints, an attempt has been made in this study to tabulate and follow the results of all blood cultures taken between July 1, 1922, and July 1, 1924.

In this paper, the following aspects are covered: The total number of blood cultures taken, with the diseases for which they were taken; the total number of positive cultures; the diseases for which these were positive, with the specific number of each and per cent of positive cultures as compared to the total number of blood cultures for each disease; total deaths for each disease; the organisms isolated for each disease; the total deaths for the organism isolated; and the end-results or the percentage of deaths in relation to positive blood cultures. The object of this paper is to record the results of blood culturing as a whole, not to follow up any one definite disease, organism, or series of cases for a comprehensive and detailed review.

During the above-stated period, there was a total of 2,092 recorded blood cultures. The diseases for which these were taken include a total of 105 different medical and surgical conditions. It should be stated here that the diagnoses recorded at the time the cultures were taken were by no means final in all cases. The culture was taken, in many instances, as an aid in the establishment of a final diagnosis. Thus, many of the cases recorded as typhoid or malaria were followed by question marks, and the diagnosis stated was the most plausible tentative one at that time. Again, it will be seen that many of the diagnoses recorded appear at first to be absurd, namely, some of the anemias, nephritis, tabes dorsalis, alcoholism, etc. These were the admitting diagnoses, but the patients subsequently developed some definite superimposed complication, with temperature, etc., which warranted taking a culture. At the head of the list of diseases for which blood cultures were taken, is typhoid fever, with 357 blood cultures; next, general sepsis,

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with 226 (not including postabortal and postpartum sepsis); lobar pneumonia, with 200; acute endocarditis, with 169; postpartum sepsis, 91; subacute bacterial endocarditis, 51; and so on. Chart 1 shows a list of all these diseases with the total number of blood cultures taken during each of the two years, with a total for the two years.

The total number of recorded *positive* cultures obtained during this time for some of the more important diseases are:

Typhoid.—Forty-one cases, or 11.2 per cent of the total cultures taken for this disease. Widals were positive in 77 cases, so that there were 53.2 *per cent of positive typhoid cultures to total positive Widals.*

General sepsis.—Thirty-nine cases, or 17.7 per cent of all cultures taken for this disease.

Lobar pneumonia.—Forty-two cases, or 21 per cent.

Acute endocarditis.—Sixteen cases or 10 per cent.

Cerebrospinal meningitis.—Fifteen cases or 45.4 per cent.

Osteomyelitis.—Seven cases or 16.4 per cent.

Postpartum sepsis.—Seven cases or 7.7 per cent.

And so on for several more or less important diseases, culturally speaking. This data is tabulated in toto on Chart 1 in columns 4 and 5. The total number of positive cultures for all diseases is 221, or 10.5 per cent of the total cultures taken. In many cases more than one positive blood culture was taken, and in these circumstances only one was used for our purposes.

It is interesting to note the number of recorded deaths for each disease and to tabulate the percentage of deaths in relation to the positive blood cultures for each disease, together with the per cent of deaths to the total cases for each disease. Thus, in typhoid fever, during this period of time, there was a total of 11 recorded deaths, or 27 per cent of the total of 41 cases with positive blood cultures, with a percentage of deaths in relation to a total cases of only 3 per cent. Even though all of the 357 cases were not typhoid fever, yet it must be granted that this percentage is remarkably low. Sepsis claimed 31 cases, or 79 5 per cent of positive blood cultures, a rather high percentage, but yet the high fatality of general septicemia, caused for the most part by the streptococci and the staphylococci, as is true in these cases, is a notorious fact. Still, this is 13.7 per cent of the total blood cultures taken for suspected sepsis, a moderately low figure. Of the 42 cases of lobar pneumonia with positive blood cultures, the majority of which were due to pneumonia, 34 died, or 81 per cent. Of course, this strikes one as being a very high figure for percentage of deaths in pneumonia, but when one realizes that these are all pneumonias with positive blood cultures and that it represents deaths which were only 17 per cent of the total 200 cases of lobar pneumonia for which blood cultures were taken, one realizes that it is not so high a mortality rate as might at first be supposed. One cannot compare this 17 per cent of deaths to the average mortality rate, which is approximately 30 per cent of deaths for all cases of pneumonia, because of the fact that all the cases of lobar pneumonia were by no means cultured, and this does not represent any definite proportion of the total pneumonias admitted to the wards.

CHART 1

BLOOD CULTURES AT BELLEVUE HOSPITAL FROM JULY 1, 1921 TO JULY 1, 1923

DISEASES	1 1921-22	2 1922-23	3 TOTAL FOR BOTH YEARS	4 NUMBER OF POSITIVE	5 PER CENT POSITIVE OF TOTAL TAKEN	6 NUMBER OF DEATHS	7 PER CENT DEATHS TO TOTAL POSITIVES	8 PER CENT DEATHS TO TOTAL CULTURES TAKEN
1. Typhoid	194	163	357	41	11.2	11	27.	3.
2. Widals, positive	37	40	77	–	53.2 of positive cultures to pos. Widals.			
3. Sepsis, general	137	89	226	39	17.7	31	79.5	13.7
4. Sepsis, postabortal	13	34	47	2	4.2	2	100.	42.5
5. Sepsis, postpartum	73	18	91	7	7.7	3	43.	3.3
6. Endocarditis, subac. bact.	–	51	51	7	13.7	4	57.	8.
7. Endocarditis, acute	125	44	169	16	10.	13	81.2	7.6
8. Endocarditis, rheumatic	–	9	9	–	–	–	–	–
9. Pericarditis, acute	2	5	7	–	–	–	–	–
10. Myocarditis, chronic	6	–	6	–	–	–	–	–
11. Chr. cardiovalvular disease	17	31	48	–	–	–	–	–
12. Pneumonia, lobar	123	77	200	42	21.	34	81.	17.
13. Pneumonia, broncho	3	6	9	–	–	–	–	–
14. Meningitis, cerebrospinal	17	16	33	15	45.4	11	73.3	33.3
15. Arthritis, infectious	17	12	29	4	13.8	1	25.	3.4
16. Arthritis, gonococcal	1	2	3	–	–	–	–	–
17. Osteomyelitis	25	18	43	7	16.4	3	43.	7.
18. Tuberculosis, in general	9	9	18	–	–	–	–	–
19. Anemias	5	9	14	–	–	–	–	–
20. Purpuras	4	5	9	–	–	–	–	–
21. Leucemias	6	–	6	–	–	–	–	–
22. Fractures	6	6	12	–	–	–	–	–
23. Abscesses	23	24	47	2	43.	–	–	–

CHART 1—CONT'D

	1	2	3	4	5	6	7	8
DISEASES	1921-22	1922-23	TOTAL FOR BOTH YEARS	NUMBER OF POSITIVE	PER CENT POSITIVE OF TOTAL TAKEN	NUMBER OF DEATHS	PER CENT DEATHS TO TOTAL POSITIVES	PER CENT DEATHS TO TOTAL CULTURES TAKEN
24. Mastoiditis	13	17	30	3	10.	1	33.3	3.3
25. Erysipelas	2	5	7	4	57.	4	100.	57.
26. Pelvic infection	1	1	2	1	50.	1	50.	100.
27. Cellulitis	10	7	17	2	11.8	2	100.	11.7
28. Anthrax	7	4	11	3	27.2	2	66.6	18.2
29. Influenza	3	3	6	1	16.6	1	100.	16.6
30. Pemphigus	–	4	4	1	25.	1	100.	25.
31. Peritonitis	5	2	7	2	29.5	2	100.	28.5
32. Empyema	10	2	12	2	16.6	1	50.	8.3
33. Carbuncle	3	3	6	1	16.6	1	100.	16.6
34. Acute rheumatic fever	14	9	23	3	39.	3	100.	13.
35. Infected hand	–	2	2	1	50.	1	100.	50.
36. Aneurysm	1	1	6	1	50.	1	100.	100.
37. Fever	5	1	6	1	16.6	1	100.	100.
38. Phlebitis	5	–	5	1	20.	1	100.	20.
39. Paratyphoid fever	3	–	3	1	33.3	–	–	–
40. Raynaud's disease	2	–	2	1	50.	1	100.	50.
41. Sinus thrombosis	17	2	19	2	10.5	–	–	–
42. Pleurisy	3	4	7	–	–	–	–	–
43. Thrombophlebitis	2	6	8	–	–	–	–	–
44. Pyelitis	7	3	10	–	–	–	–	–
45. Encephalitis	8	7	15	–	–	–	–	–
46. Undiagnosed	60	39	99	–	–	–	–	–
47. Not stated	50	75	125	–	–	–	–	–
48. Miscellaneous	75	82	157	–	–	–	–	–
Total or average			2092	221	10.5	137	62.2	6.54

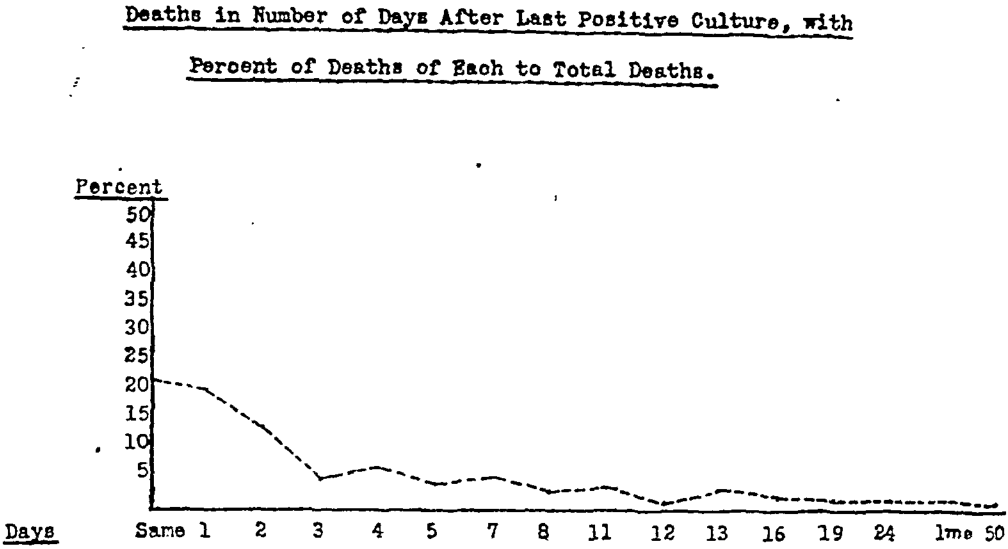


Chart 1.—Continued.

Of the 16 cases of acute endocarditis, 13 died, or 81.2 per cent. It is a well-known fact that acute or malignant endocarditis is highly fatal, so this high figure is not surprising. The percentage of deaths in relation to the total 169 cases of acute endocarditis for which cultures were taken is 7.6, a fact in itself which assures one that these cases of endocarditis either were not in their acute stage or were not endocarditis at all.

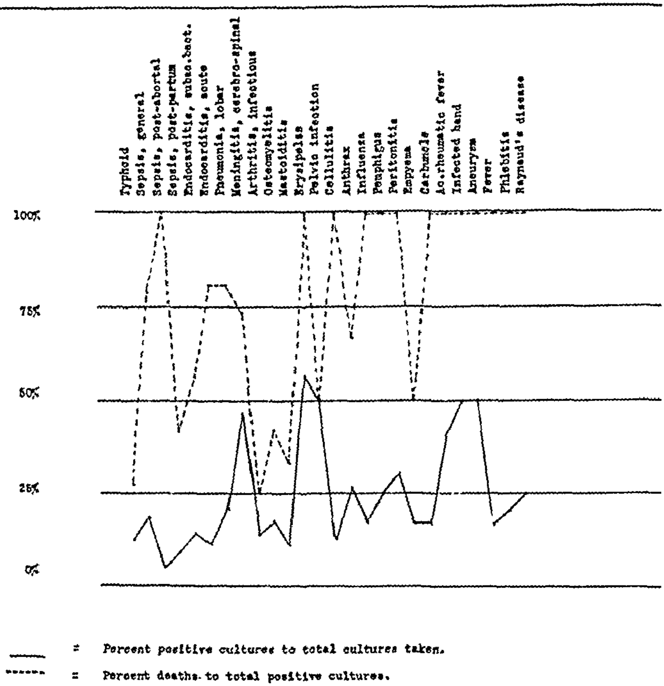
Eleven of the 15 cases with cerebrospinal meningitis died, or 73.3 per cent. This amounts to 33.3 per cent of the total 33 cases diagnosed as such. Both these figures are high, due mainly to the fact that over half (8) of these cases with positive blood cultures had pneumococci isolated; 5 had Staphylococcus aureus, while one each had meningococci and Streptococcus hemolyticus. It is fair to assume that those cases of meningitis with pneumococcus septicemia had pneumococcus meningitis, and the high mortality rate of this is well known. One outstanding fact is that, in this series of cases, only one had meningococcus septicemia. It is probable that the blood culture here was taken early in the disease when a meningococcus septicemia exists in Meningococcus meningitides.

There were 3 deaths of the 7 cases of osteomyelitis with positive blood cultures, or 43 per cent, making a death rate of only 7 per cent of the total 43 cases with osteomyelitis. This is as small a number as can be expected, especially in view of a constant focus of blood infection, which an osteomyelitis affords.

Of the 7 cases of erysipelas that were cultured, 4 were positive, and all four cases died, giving a 100 per cent mortality of the positive cultures, or a mortality of 57 per cent of all that were cultured. Two of these had Streptococcus hemolyticus, one streptococcus, and the fourth, Staphylococcus aureus.

CHART NO. 2.

Percent Positives to Total Cultures and Percent Deaths to Total Positives.



There were 11 cases of anthrax that were cultured, 3 of which were positive, or 27.2 per cent. Two of these died, giving a mortality of the total cultured of 18.2 per cent.

It is deemed unnecessary to enumerate the number of deaths with percentages of the other diseases, the results in tabulation being found on Chart 1, Columns 6, 7, and 8.

The proportion of deaths from all cases of septicemia in total positive blood cultures was 62.2 per cent. The proportion of known deaths from septicemia to total blood cultures taken was 6.54 per cent.

Generally speaking, it may be said that a large number of blood cultures are necessary and justifiable in certain diseases, especially as an aid to diagnosis. This is true in most diseases which yield a comparatively high percentage of positive blood cultures in relation to the total taken, and may be said to include general sepsis, lobar pneumonia, cerebrospinal meningitis, erysipelas and others. There is the additional fact, however, that the number

CHART 3

DISEASES WITH POSITIVE BLOOD CULTURES AND ORGANISMS ISOLATED

DISEASES	NO. CASES	ORGANISMS	NO. CASES
1. Typhoid	41	B. typhosus	38
		Streptococcus	2
		Staph. aureus	1
2. Infectious arthritis	4	Strept. viridans	1
		Staph. hemolyticus	2
		Strept. hemolyticus	1
3. Erysipelas	4	Strept. hemolyticus	2
		Staph. aureus	1
		Streptococcus	1
4. Acute endocarditis	16	Strept. hemolyticus	4
		Gonococcus	1
		Strept. viridans	6
		Staph. hemolyticus	1
		Pneumococcus	2
		Staph. aureus	2
5. Subac. bact. endocarditis	7	Streptococcus	1
		Strept. viridans	5
		Meningococcus	1
6. Sepsis, general	39	Staph. aureus	14
		Strept. hemolyticus	11
		B. coli	5
		Streptococcus	1
		Strept. viridans	1
		Staph. albus	2
		Pneumococcus	4
		Colon-typhoid group	1
7. Sepsis, postabortal	2	Streptococcus	1
		Strept. hemolyticus	1
8. Sepsis, postpartum	7	Staph. aureus	1
		Strept. hemolyticus	6
9. Pneumonia, lobar	42	Pneumococcus	33
		Strept. hemolyticus	5
		Staph. aureus	3
		Strept. viridans	1
10. Meningitis, cerebrospinal	15	Staph. aureus	5
		Pneumococcus	8
		Strept. hemolyticus	1
		Meningococcus	1
11. Osteomyelitis	7	Staph. aureus	4
		Strept. hemolyticus	2
		Staph. albus	1
12. Acute rheumatic fever	3	Staph. aureus	2
		Strept. viridans	1
13. Cellulitis	2	Staph. aureus	2
14. Anthrax	2	B. anthracis	2
15. Aneurysm	1	Strept. hemolyticus	1
16. Phlebitis	1	Strept. hemolyticus	1
17. Fever	1	Strept. hemolyticus	1
18. Carbuncle	1	Staph. aureus	1
19. Empyema	2	Staph. albus	1
		Pneumococcus	1
20. Sinus thrombosis	2	Staph. albus	1
		Strept. hemolyticus	1
21. Pelvic inflammation	1	Streptococcus	1
22. Raynaud's disease	1	Strept. hemolyticus	1
23. Mastoiditis	3	Pneumococcus	2
		Strept. hemolyticus	1

CHART 3—CONT'D

DISEASES	NO. CASES	ORGANISMS	NO. CASES
24. *Peritonitis*	2	Pneumococcus	1
		Strept. hemolyticus	1
25. Pemphigus	1	Staph. aureus	1
26. *Abscess*	2	Strept. hemolyticus	1
		Staph. aureus	1
27. Influenza	1	Streptococcus	1
28. Paratyphoid fever	1	B. paratyphosus	1

CHART 4

ORGANISMS ISOLATED WITH DEATHS AND PERCENTAGE DEATHS FOR EACH

	NO. CASES	NO. DEATHS	PER CENT DEATHS TO CASES
1. Pneumococcus	51	38	72.5
2. Streptococcus hemolyticus	42	26	61.9
3. B. typhosus	38	11	28.9
4. Staphylococcus aureus	38	31	80.
5. Streptococcus viridans	15	10	66.6
6. Streptococcus	8	8	100.
7. B. coli	5	4	80.
8. Staphylococcus albus	5	3	60.
9. Staphylococcus hemolyticus	2	2	100.
10. Meningococcus	2	1	50.
11. B. anthracis	3	2	66.6
12. Colon-typhoid group	1	0	–
13. Gonococcus	1	1	100.
14. B. paratyphosus	1	0	–
Total	221	137	72.2 (average per cent)

CHART 5

DEATHS IN NUMBER OF DAYS AFTER LAST POSITIVE BLOOD CULTURE

	NO. OF DAYS	NO. OF CASES	PER CENT TO TOTAL DEATHS
1.	Same day	31	22.6
2.	1 day	29	21.1
3.	2 days	20	14.6
4.	3 "	7	5.1
5.	4 "	10	7.3
6.	5 "	6	4.3
7.	7 "	7	5.1
8.	8 "	4	2.9
9.	11 "	5	3.6
10.	12 "	1	.7
11.	13 "	4	2.9
12.	16 "	3	2.1
13.	19 "	3	2.1
14.	24 "	3	2.1
15.	1 month	3	2.1
16.	50 days	1	.7
	Total	137	

of unnecessary and sterile blood cultures can be lessened, particularly from a diagnostic point of view, in many diseases yielding a low percentage of positives in relation to the total taken, such as typhoid, postabortal sepsis, postpartum sepsis, acute endocarditis, acute rheumatic fever, mastoiditis, etc., and others in which no positive blood cultures were obtained at all, such as chronic cardiovalvular disease, the tuberculous affections, fractures, empyema.

It may be said that if it is thought that a possible positive blood culture will aid in the establishment of a known diagnosis from an unknown, that it is justifiable, whatever the circumstances. Chart 2 shows graphically the *percentage of positive cultures in relation to total* cultures, and the percentage of deaths in relation to total positive cultures.

The diseases in which positive blood cultures were obtained totaled 28, or 26.6 per cent of the total number of conditions for which they were taken. These diseases with positive blood cultures with organisms isolated for each disease and percentage of each organism in relation to the total for the disease will be found on Chart 3.

It is interesting to study the end-results of septicemias according to the organisms isolated from blood cultures. There was a total of 51 cases with cultures positive for pneumococci; of which 38 or 72.5 per cent died. Most of these had lobar pneumonia. It has been seen that the percentage of deaths in relation to positive cultures in this disease was 81 per cent, so that the figures for the pneumococcus septicemias are somewhat better as far as end-results are concerned. There were 42 cases of positive cultures of Streptococcus hemolyticus. Of these, 26 or 61.9 per cent died. The high death rate of septicemia from this organism is well known, and it can be safely said that this is about as low a percentage for Streptococcus hemolyticus septicemia as can be found. It is evident that transfusions and blood antiseptic solutions are still entirely ineffective in the majority of instances. Of the 38 cases of typhoid with positive cultures of B. typhosus, 11 or 28.9 per cent died. There were 38 Staphylococcus aureus septicemias, with 31 deaths or 80 per cent—a high figure, as is expected. Most of these for any one disease occurred in general sepsis, where 14 cases had Staphylococcus aureus septicemia. Fifteen cases had Streptococcus viridans septicemia, with death claiming 10 of these, or a high mortality of 66.6 per cent. There were 8 cases of positive streptococcus blood cultures, with 8 deaths, or 100 per cent mortality. A high rate is to be expected, but one as high as this seems to be too high, though it may be mentioned that if more cases of streptococcus septicemia were included, some cases would undoubtedly have been found with recovery. The remainder of the organisms isolated with number of deaths and percentage of deaths in relation to positive cultures are to be found in Chart 4.

A tabulation has been made ascertaining the number of days that elapsed from the date of the initial positive blood culture to the date of death or discharge from the hospital. Of course, this is not so valuable in the cases that were discharged, for in many cases the patients were discharged unimproved against the orders of the physician in charge, and may have died at home, so that they cannot be brought into our records. Even in the cases with positive blood cultures, the number of days following a positive culture that elapsed before death occurred does not necessarily indicate the seriousness of the disease, as a patient might have had a septicemia for days before being brought into the hospital and may have died soon after the positive culture was obtained.

Of the 221 cases with positive blood cultures, there were 137 known deaths, or 62.2 per cent of the total positive cultures. The greater number of deaths for any one day occurred on the same day that the positive culture was taken, less on the day after, and so on. Thirty-one died on the same day as the positive culture was taken, 29 on the day after, 20 two days after, 7 three days after, 10 four days after, 6 five days after, etc., the number of deaths being in inverse ratio to the number of days after the last positive culture was taken. The number of days after positive culture that death occurred is found on Chart 5.

SUMMARY

1. A total of 2,092 recorded blood cultures, taken during two years, was studied and the final end-results tabulated. There were 105 different medical and surgical conditions for which blood cultures were taken. Many cultures were taken to aid in the establishment of a diagnosis. The greatest number of cultures were taken from patients of suspected typhoid fever (357 cultures), with a small progressive number from cases of sepsis, lobar pneumonia, acute endocarditis and others.

2. Altogether 221 positive blood cultures were obtained (10.5 per cent of the total cultures taken).

3. The proportion of deaths from all cases of septicemia to total positive blood cultures was 62.2 per cent. The proportion of known deaths from septicemia to total blood cultures taken was 6.54 per cent.

4. The diseases with positive blood cultures totalled 28, or 26.6 per cent of the total number of conditions for which they were taken.

5. The organisms isolated include the usual pathogenic ones, the majority of positive cultures being composed of pneumococci, Staphylococcus aureus, B. typhosus, Streptococcus hemolyticus and Streptococcus viridans.

6. The percentage of deaths in relation to total positive cultures for each organism range from 100 per cent with gonococci, Staphylococcus hemolyticus and streptococci to 61.9 per cent for Streptococcus hemolyticus, 50 per cent for meningococci, and 28.9 per cent for B. typhosus. The average percentage of deaths in relation to total positive cultures for all organisms was 72.2 per cent.

7. The majority of deaths occurred on the same day or within four days after the first blood culture was taken.

A LABORATORY STUDY OF AN EXTENSIVE EPIDEMIC OF SEPTIC SORE THROAT*

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IN EPIDEMICS of so-called septic sore throat which have been investigated previously, a hemolytic streptococcus has generally been considered the etiologic agent. Such reports are so numerous and well known that a review of the literature is unnecessary. An epidemic of 500 cases of septic sore throat occurring in a village of approximately 3,500 inhabitants during May and June of 1925† offered an opportunity to apply the methods now available for investigating such infections. It was thought of interest, moreover, to determine the relationship of the hemolytic streptococci present to those isolated from other infections, especially those from scarlet fever, and also to exclude the possible presence of hitherto unsuspected bacterial incitants.

The clinical aspect of the acute cases resembled that described by other workers. The onset was usually sudden with headache, chills, muscular soreness, and diffuse redness and swelling of the throat. In some cases, the cervical glands were swollen and tender. No rash was noted in any case, and no desquamation was reported.

An intensive bacteriologic examination was made of a few of the acute cases. Swabbings from the throats were cultured directly on various kinds of media, animals were inoculated, and preparations were made for microscopic examination. The blood was cultured in two instances, but no growth of bacteria was obtained. The microscopic and cultural examinations of the throat swabbings showed hemolytic streptococci to be the predominating organisms. Rabbits inoculated intrapleurally with throat swabbings from one of the patients whose blood was cultured and from one other acute case, died after six and sixteen days, respectively. Pure cultures of hemolytic streptococci were recovered from their blood and from pleural abscesses. Rabbits inoculated intravenously with 0.5 c.c. and 1 c.c. of broth cultures of the streptococci recovered from the first rabbits, failed to show any signs of infection. These cultures proved fatal for mice in 0.2 c.c. amounts, but the virulence was not increased by passage through 16 of these animals. In an attempt to rule out the possibility of infection with *B. pneumosintes,* 2 rabbits were inoculated intratracheally with the same throat swabbings as those used for the intrapleural inoculations. These animals showed no abnormalities in blood counts, nor any evidence of a pneumosintes infection when killed forty-eight hours later. Further passage by means of intratracheal inoculation of lung tissue failed to induce any lesions in rabbits.

*From the Division of Laboratories and Research, New York State Department of Health, Albany.

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†An epidemiologic study of the outbreak was made by Dr. B. E. Roberts, Epidemiologist of the State Department of Health, whose report will be published separately.

Since the clinical aspect of septic sore throat is not unlike that of scarlet fever, it was thought advisable to determine the relation of the streptococci isolated from these cases to streptococci from scarlet fever by studying the toxin production and serologic reactions of a number of strains.

TOXIN PRODUCTION

Culture filtrates of 12 strains isolated from acute convalescent and recovered cases were tested intracutaneously on goats which were known to be susceptible to a standard scarlet fever streptococcus toxin. Under the conditions of the test, 6 of the strains produced no toxin. Five other strains produced toxins of very low potency, 4 of which were neutralized by an immune goat serum produced against the standard strain of scarlet fever streptococcus, while the fifth toxin was not completely neutralized in the dilutions tested. In the case of 2 strains, successive passage through 15 mice failed to increase their virulence or toxicity.

One strain isolated from a recovered case produced a potent toxin neutralized by the immune goat serum. This patient had no previous history of scarlet fever. His serum neutralized the toxin produced by the strain isolated from his throat and also the standard scarlet fever streptococcus toxin. The patient did not react to the standard scarlet fever streptococcus toxin; but the significance of this result is doubtful, since no test had been made previously.

During July and August the intracutaneous test for susceptibility to scarlet fever streptococcus toxin was made on a group of 20 persons who had had definite cases of septic sore throat during the epidemic, although cultures had been submitted from only 6 of them. It was thought that, if an unusually large proportion of persons failed to react to the toxin, it would indicate that this immunity might have been acquired as a result of the recent infection with streptococci. In this small series the findings were not suggestive, since 30 per cent of those tested reacted definitely to the toxin, which is approximately the same percentage as that found in a group of normal individuals of the same ages (eight to fifty years).

AGGLUTINATION AND PRECIPITIN TESTS

Seven of the strains were tested for agglutination in rabbit serums immune to the Dochez strain of scarlet fever streptococcus. Homogeneous suspensions were obtained for these tests by transferring the cultures daily for two weeks in pneumococcus broth. The tests were made in dilutions ranging from 1:40 through 1:1280 (the titer of the serum) and were read after being incubated for two hours at 37° C. and again after standing overnight in the refrigerator. Five of these strains were agglutinated in the 1:1280 dilution of the serum, one giving a much less definite reaction than the others. The other 2 strains tested were agglutinated in the 1:80 and 1:160 dilutions of the serum, respectively.

An extract prepared by heating the sediment from an eight-day broth culture of the Dochez strain of scarlet fever streptococcus, gave a definite precipitin reaction with the homologous immune rabbit serums used for the

agglutination tests. Precipitinogens prepared in the same way from 4 of the strains of streptococci, 1 being the toxin producer, gave equally definite reactions with these serums.

SUMMARY

Hemolytic streptococci were found to be the predominating organisms in throat cultures from the acute cases studied in an extensive epidemic of septic sore throat. The toxicity of 12 strains was tested. One produced a potent toxin neutralized by our standard scarlet fever antistreptococcus serum and also by the patient's serum. The other 11 produced little or no toxin. The precipitinogens and agglutinating antigens prepared from 4 of the nontoxic strains could not be distinguished from those prepared from the 1 toxic strain nor from the Dochez strain of scarlet fever streptococcus.

Among a group of 20 convalescents, 30 per cent gave definite skin reactions to a standard scarlet fever streptococcus toxin, which is approximately the same proportion as in a group of normal individuals of the same age (eight to fifty years).

LABORATORY METHODS

A SIMPLE METHOD FOR CULTIVATING ANAEROBES BY MEANS OF PHOSPHORUS*

By P. L. Varney, M.S., St. Louis, Mo.

DURING a course of study of anaerobic mouth organisms, it became necessary to devise some method whereby large numbers of tube and plate cultures of these organisms could be quickly and economically placed under strictly anaerobic conditions with no danger of contamination of the cultures at any stage of the process.

Numerous methods for anaerobic tube and plate cultures are to be found in the literature, but they all have some objection because of which they have been discarded for a better method. With certain of these methods, the apparatus is left in a sticky, blackened condition, and an undue amount of labor is necessitated when large numbers of cultures must be incubated and examined. The various pyrogallate methods, including that of Wright's, which had been used previously in this work, are open to this objection. Others require expensive and complicated apparatus or chemicals, are not certain in action, require constant watching, are dangerous to use, or allow possible contamination during incubation or removal of the plates or tubes from the apparatus.

The simplest and best of the various methods are those which depend on the removal of oxygen, by reduction or displacement, from large containers in which the inoculated material has been placed. Of these methods, those which depend on the oxidation of metallic phosphorus for the removal of the oxygen seemed to me to offer the greatest possibilities in economy, safety, and ease of operation.

Sellards,[1] the first to use such a method, devised an apparatus whereby he secured anaerobiosis in both plates and tubes. His apparatus was cumbersome, however, and consumed too much time in operation. Bushnell[2] modified this method by using an aluminum pressure cooker for the anaerobic chamber, securing anaerobiosis by burning phosphorus within the tightly sealed chamber. While rapid and economical, this also requires a piece of apparatus which is found in but few laboratories; hence, it has a limited range of use.

In the method described, the apparatus has been so designed that it can be made from inexpensive material to be found in all laboratories, or that can be readily constructed.

The apparatus, as shown in Fig 1, consists of a standard museum jar (*H*), into which is fitted a heavy wire frame (*G*), to hold the Petri dishes or tubes.

*From the Department of Bacteriology and Public Health, Washington University School of Medicine.

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The museum jar may be of any desired size. For Petri dishes, the five by twelve inch size is very satisfactory, accommodating from 10 to 15 plates of 10 mm. depth. The jar must not leak. It may be supplied with a closely fitting rubber gasket, or the lid may be ground in place, thus doing away with the necessity for a gasket.

The frame (*G*) is constructed of heavy steel wire, which is preferably about one-eighth inch in diameter. It is made just large enough to fit in the museum jar without jamming. It must be of such a height that the top of the frame reaches no higher than the bottom of the heavy flange about the mouth of the jar. About one inch from the bottom of the frame is soldered a platform, made preferably of galvanized wire gauze. When placed within the frame, the Petri dishes (*E*) rest on this platform and are thus kept out of the water which is used in the jar.

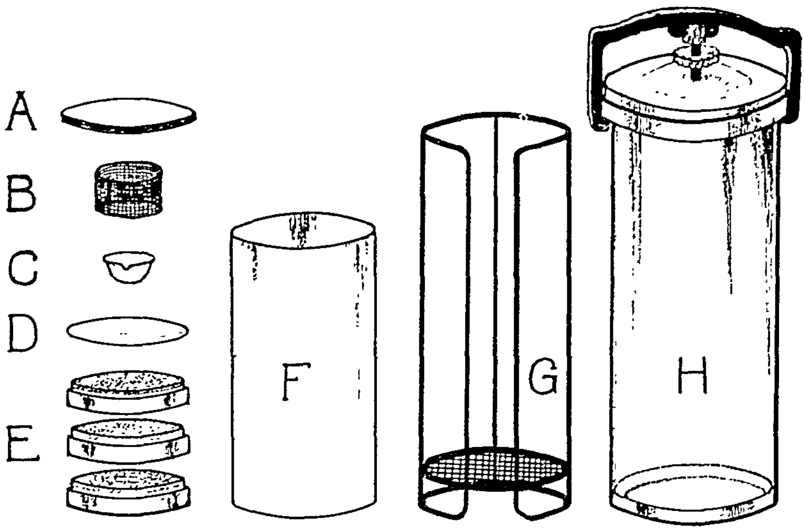

Fig. 1.

After the Petri dishes have been placed within the frame, a cylinder (*F*), made either of paper or tin, is placed over them. This is done to protect the sides of the Petri dishes or tubes from the phosphorus pentoxide vapors which form during the combustion of the phosphorus. The glassware is thus kept free from all deposit. A less satisfactory method of accomplishing the same result is to place a piece of filter paper (*D*) over each plate. If the cylinder is of tin, it should be closed at the top.

When the plates have been loaded into the frame, the tin cylinder is placed over them, and on top of this, resting on it, is placed a dish for holding the phosphorus. This may be of tin or porcelain. A small evaporating dish answers the purpose admirably, as does an empty ether can, cut in half. About this container is placed a screen (*B*), made of iron or nichrome wire gauze, which protects the sides of the museum jar from the burning phosphorus. A piece of asbestos board is cut to form a disc (*A*) of such size that it will fit on top of this gauze, and slip up and down freely within the frame. This permits of the use of a few or of many Petri dishes with the disc tightly in place over the gauze frame. In addition to the asbestos disc over the

screen, it is well to have another which rests on top of the frame, fitting snugly against the jar. This is an added factor of safety against the breakage of the jar or lid.

When the apparatus is assembled ready for use, about one-half inch of water is placed in the bottom of the jar. The water absorbs the phosphorus pentoxide vapor, formed during the combustion of the phosphorus. The Petri dishes or tubes are placed within the frame, and the tin or paper cylinder is placed over them. With the plates, evaporating dish, gauze, and asbestos disc in place, test the apparatus to see that the lid will fit tightly. Now thoroughly lubricate the mouth of the jar and the apposing surface of the lid with stopcock lubricant or a stiff mixture of vaseline and paraffin. It is also well to lubricate the rubber gasket. The latter should be put in place on the lid of the jar before loading the chamber with phosphorus.

To secure anaerobic conditions, place within the evaporating dish a piece of yellow stick phosphorus about one-eighth to one-quarter of an inch long. This is a sufficient charge for the five by twelve inch jar. Quickly place over the dish the asbestos disc (*A*), and see that it fits down snugly against the gauze surrounding the dish. Now clamp the lid into place. Within a few seconds the phosphorus takes fire and burns violently, establishing anaerobic conditions in the jar within a very few seconds. The heat given off by the oxidation of the phosphorus raises the temperature of the jar to approximately 40° C., so that an incubating temperature suitable for most anaerobes is quickly reached. This temperature will be maintained, with but a slight drop, if the apparatus is now put in the incubator. Left at room temperature, however, the heat is quickly dissipated, and the temperature drops to that of the room. When gelatin plates are to be used in the apparatus, if one first thoroughly chills the jar before adding the phosphorus, the final temperature within the chamber will not rise to a point sufficient to melt the gelatin.

This method has been used successfully in the cultivation of numerous mouth anaerobes for over a year, and no trouble has been experienced. It has been used successfully in the cultivation of many obligate anaerobes, very good results being secured in the case of the intestinal organisms. Surface colonies grow very well, and these may be examined under the microscope for colony form, thus facilitating diagnosis. Gelatin dilution plates are readily incubated in this apparatus, any evidence of liquefaction being observed in the original plate.

Certain precautions must be observed in the handling of the phosphorus to avoid premature combustion and danger of burns. Keep it in cool water at all times, being especially careful of this detail in summer. Unless this precaution is taken, burns or jar breakage may result. It is well to keep the phosphorus in the ice chest, wherever possible. Handle the material with forceps only, first making sure that they are cool. When opening the anaerobic chamber after incubation, immediately flood the unburned phosphorus with water, or remove the combustion dish to the hood with forceps. Any excess phosphorus may be recovered by melting under warm water, then

cooling. If the above precautions are taken, there will be no danger from burns or from phosphorus fumes.

Danger of breakage to the jar is obviated by the construction of the protecting screen and asbestos discs. Although the apparatus has been used more than a year by several bacteriologists, no breakage has occurred.

The moisture content within the jar is very high during incubation, thus aiding the growth of bacteria. Because of this, however, certain precautions must be taken to prevent the dropping off of the labels. These may be painted over with melted paraffin, or the articles may be marked with a blue wax pencil. India ink may also be used to mark the plates, but this is not as satisfactory as the wax pencil.

No trouble has been experienced with the phosphorus fumes injuring the growth. The most delicate anaerobes grow readily. A slight amount of elemental phosphorus is liberated, but if the plates are covered as described, this does not reach them. It has, however, no injurious effect on bacterial growth, as has been demonstrated not only from our own experience but also from that of Sellards and Bushnell as well.

This method possesses certain advantages:

1. All obligate anaerobes, even the most delicate, grow readily.

2. It is economical; the average cost of the phosphorus used for a dozen plates is from one-half to one cent.

3. It is rapid and certain. Anaerobic conditions are very quickly established, and if a slight leak occurs, the excess phosphorus will oxidize and completely remove all oxygen.

4. Both liquid and solid media cultures may be incubated in the apparatus. Loop dilution plates may be readily prepared.

5. There is no danger of contamination of the cultures at any stage of the process since the plate or tube remains unopened until ready for examination.

6. It retains its anaerobic conditions indefinitely and may be used for the storing of surface cultures.

7. By decreasing the amount of phosphorus used it may be used for the cultivation of organisms requiring a reduced oxygen tension.

8. A series of plates or tubes may be incubated under identical physical conditions, giving a good basis for comparison of cultures.

SUMMARY

A new anaerobic method, depending on the oxidation of yellow phosphorus, has been described. In relation to its safety, ease of operation, certainty and cleanliness, it is superior to other methods. All anaerobes may be successfully grown, either in liquid or solid cultures, without danger of contamination. The average cost of incubation is less than one-twentieth of a cent per plate.

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[1]Sellards, A. W.: Some Researches on Anaerobic Cultures with Phosphorus, Zentralbl. f. Bakteriol., I. O., 1904, xxxvii, 632-637.

[2]Bushnell, L. D.: A Method for the Cultivation of Anaerobes, Jour. Bact., 1922, vii, 277-281.

THE BOLTZ TEST: A TEST OF PRACTICAL VALUE IN THE DIAGNOSIS OF GENERAL PARALYSIS*

By Oswald H. Boltz, M.D., New York

IN 1923, I first described the above test under the title: "Studies on the Cerebrospinal Fluid with an Acetic Anhydride-Sulphuric Acid Test,"[1] and called attention to the frequency of a positive reaction with it upon the spinal fluids of certain cases of neurosyphilis, especially in general paralysis. Since then, however, two English observers have worked with the same test and have not only confirmed my investigations, but have also stressed the value of the test as a diagnostic aid in general paralysis. Both drew attention to the ease and rapidity with which our test could be performed, and, as a result of their findings, attested to its reliability in the diagnosis of general paralysis.

THE METHOD OF PERFORMING THE TEST

Place 1 c.c. of fresh cerebrospinal fluid in a small glass test tube and to it add 0.3 c.c. of acetic anhydride. Shake the mixture well and then add, drop by drop, 0.8 c.c. of concentrated sulphuric acid. Shake the mixture gently once more. Then hold the test tube against a white background. The presence of a lilac tint indicates a positive reaction; a brown yellow, red yellow, or clear fluid is noted if the reaction is a negative one. The lilac color, characteristic of the positive reaction, may appear immediately after the addition of the sulphuric acid and, in slightly positive cases, may disappear in a minute or two; therefore one should watch the cerebrospinal fluid closely as soon as the sulphuric acid has been added, or a slightly positive case may be missed. In the majority of cases the lilac tint will remain for several minutes, occasionally for several hours.

The chemistry involved in the test, so far as I know, is not understood and awaits the research of a physiologic chemist.

In my original paper upon the subject, I concluded that our test was positive in all cases of general paralysis; that among psychiatric cases, it was predominantly positive in cases of neurosyphilis; that in psychoses not due to or complicated with syphilis, and of a functional nature, the test was negative; and that it differentiated two groups of tabetics: one giving a negative reaction, the other showing only a slightly positive one. In four cases of neurosyphilis with a negative spinal fluid Wassermann reaction, our test was positive.

In 1925, Grossman,[2] comparing the diagnostic merits of the Wassermann reaction with other simple tests used in the diagnosis of neurosyphilis, examined 60 cases which included 28 cases of general paralysis and one case of

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locomotor ataxia. Using our test, he found that it was positive in all of his group of general paralytics, and negative in the one case of locomotor ataxia, as well as in the remainder of cases consisting of epileptics, psychoses due to arteriosclerosis, senile dementia, dementia precox, cases of melancholia, mania, and psychoses due to alcoholism. Grossman also found that the more active and advanced the syphilitic condition of the nervous system, the more positive was our test.

In 1926, Harris,[3] of the West Park Mental Hospital in Epsom, England, published an article in which he advocated the use of our test as a rapid, simple, and reliable test for confirming a clinical diagnosis of general paralysis. He called attention to the fact that the diagnosis of this disorder depends upon the recognition of certain mental symptoms, the eliciting of a number of physical signs, and the finding of certain definite changes in the cerebrospinal fluid. The serologic changes in the spinal fluid usually looked for to confirm a clinical diagnosis of general paralysis are: (*1*) a positive Wassermann reaction; (*2*) a paretic type of Lange colloidal gold curve (5555443211); (*3*) the presence of an increased amount of globulin; (*4*) a pleocytosis. Harris quite justly points out that, while a cell count and a globulin test on the spinal fluid can be performed with ease and rapidity and are of greatest value to the clinician who is often called upon to make a quick diagnosis, they alone do not furnish conclusive proof of the presence of general paralysis; that delay occurs until a laboratory report on the Wassermann reaction and the Lange colloidal gold curve are at hand. And furthermore, he believes that the discovery of a test which could be easily and quickly performed and which would be as reliable as the Wassermann reaction would be of considerable importance to the clinician, whether he were a consulting physician or a general practitioner. Using our test and comparing it with other serologic methods, Harris tabulates the results of his investigation in 180 cases as follows:

Of 92 cases of general paralysis the cerebrospinal fluid showed:		
Wassermann reaction positive	92 =	100 per cent
Boltz test positive	89 =	97 per cent
Lange curve of paretic type	84 =	91 per cent
Increased amount of globulin	90 =	98 per cent
Increased number of cells	91 =	99 per cent
Of 5 cases of neurosyphilis (not general paralysis) the cerebrospinal fluid showed:		
Wassermann reaction positive	5 =	100 per cent
Luetic Lange curve	4 =	80 per cent
Boltz test positive	2 =	40 per cent
Increased amount of globulin	4 =	80 per cent
Increased number of cells	5 =	100 per cent
Of 83 cases of other types of mental disorder the cerebrospinal fluid showed:		
Wassermann reaction positive	0 =	0 per cent
Boltz test positive	1 =	1 per cent
Lange curve of positive type	1 =	1 per cent
Increased amount of globulin	5 =	6 per cent
Increased number of cells	1 =	1 per cent

The above table shows that of 92 cases of general paralysis, a positive reaction with our test was obtained in 97 per cent. Harris states: "Of the three exceptions one was an undoubted case of general paresis with the typical mental symptoms, well marked physical signs, and positive cerebro-

spinal fluid findings. The other two cases, however, were by no means typical, and were probably very early cases in which there were few mental changes, some physical signs, and a slightly positive Wassermann reaction in the cerebrospinal fluid. In another case, a well-marked general paretic, the Wassermann reaction of the cerebrospinal fluid and the Lange curve were found to be negative at the first examination, but the acetic anhydride-sulphuric acid test (Boltz Test) was strongly positive, and the number of cells and the amount of globulin were slightly increased. A second specimen of fluid was examined a fortnight later, and this time a very faintly positive Wassermann reaction (+3) was obtained, the cells and globulin were slightly increased, the Lange test was negative, and the acetic anhydride-sulphuric acid test was again strongly positive. In this case, therefore, the acetic anhydride-sulphuric acid test (Boltz Test) was more sensitive than the Wassermann reaction." The same author found that the degree of positivity of our test in cases of general paralysis does not vary directly with that of the Wassermann reaction; that in a number of paretics who had undergone a course of malarial treatment our test showed no change, whereas the Wassermann reaction had in most cases become less strongly positive; that it appears to be more sensitive and reliable in general paralysis than the Lange colloidal gold test, since a positive result with it was obtained in 97 per cent of these cases, as against 90 per cent in the case of the Lange test. Of the 83 cases of other types of mental disorder not attributed to syphilis, only one case, a patient suffering from senile dementia, gave a faintly positive reaction with our test.

In a summary of the results of his investigations with the Boltz Test, Harris feels that it would seem justifiable to draw the following conclusions:

1. The Boltz Test upon the cerebrospinal fluid of cases of general paralysis is positive in almost every case (97 per cent), and hence is almost as reliable as the Wassermann reaction in this condition. *2.* It is negative in almost every other type of mental disorder except certain cases of neurosyphilis (other than general paralysis of the insane). *3.* It is a test which can be performed with great ease and rapidity and hence is of definite value to the clinician.

Both Grossman and I feel that the more active and advanced the syphilitic condition of the nervous system, as is the case in general paralysis, the stronger will be the reaction obtained with our test. In this organic disease, the nervous system is attacked from top to bottom by the Treponema pallidum and its destructive influence. It would seem, therefore, that the Boltz Test is an index of the destruction effected by the Treponema pallidum upon the tissues of the central nervous system. One might even be justified in assuming, perhaps, that it is of prognostic value in cases of neurosyphilis other than general paralysis. Of 21 cases of neurosyphilis (not general paralysis) in my series, 6 cases gave a slightly positive, 2 cases a positive, and 1 case a strongly positive reaction with our test, i.e., the test was positive in 42 per cent of neurosyphilitics who were not paralytics. In 5 cases of the same type studied by Harris, 2 cases (40 per cent) gave a positive reaction. To my mind it is quite possible that in those cases of neurosyphilis (not general paralysis) which gave a slightly or strongly positive Boltz Test, the

prognosis, from the standpoint of therapeutics, is not as good as in those who give a negative reaction. For the present, however, the prognostic value of the test still remains a problem to be checked up in clinics devoted to the treatment of neurosyphilis.

REFERENCES

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[2]Grossman: Jour. Ment. Sc., July, 1925, lxxi, 439.
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DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

Lorber, J. A.: Simple Micro-Method for the Determination of Sugar in the Blood. München. Med. Wchnschr., November, 1925, lxxii, 1921.

1. To 0.3 c.c. of serum or blood in a small centrifuge tube add 1.7 c.c. of distilled water and 0.5 c.c. of 10 per cent sodium tungstate.
2. Add 0.5 c.c. ⅔ N sulphuric acid.
3. Centrifuge and transfer 1.5 c.c. of the clear fluid to a graduated centrifuge tube.
4. Add 1 c.c. of Fehling's solution and "cook with steam" for 90 seconds.
5. Centrifuge, remove the supernatant fluid and to the residue of red cuprous oxide add 0.3 c.c. of nitric acid, 1 or 2 drops of hydrogen peroxide, and 0.2 to 0.3 c.c. of ammonium hydroxide.
6. Dilute to 1 or 1.5 c.c.
7. The copper-ammonium solution thus formed is compared with a similar standard solution of known concentration.

Pinner, M.: The Daranyi Flocculation Reaction in Pulmonary Tuberculosis. Am. Rev. Tuberc., December, 1924, x, No. 4, p. 441.

To 0.2 c.c. of serum add 1.1 c.c. of diluted alcohol (1 c.c. of 96 per cent alcohol to which has been added 4 c.c. of 2 per cent NaCl solution).

Mix and place in a water-bath at 60° C. for 20 minutes. Readings of the degree of flocculation are made at ½, 1, 2, 3 and 24 hours.

Flocculation in one-half to 1 hour is termed plus-four; after 2 hours, plus-three; after 3 hours, plus-two; and after 24 hours, plus-one.

A study of the reaction in 534 patients is reported, from which it is concluded that the reaction has little diagnostic value but appears to be of use in indicating the severity of the destructive process.

Henley, R. R.: The Influence of Iron on the Growth of the Tubercle Bacillus upon Glycerinated Broth. Am. Rev. Tuberc., November, 1925, xii, No. 3, p. 246.

During the course of experiments upon the possible inhibitory action of metals in metallic form upon the growth of B. tuberculosis, a striking difference was noted in the growth in media containing iron, the pellicle being heavier, more wrinkled, and more tenacious.

The medium used by the author as a routine for the culture of B. tuberculosis is a beef-infusion broth containing 1 per cent of peptone, 0.5 per cent of potassium biphosphate, and 7 per cent of glycerin by weight.

The reaction is adjusted with 2/N NaOH so that 100 c.c. of the medium requires 0.6 c.c. N/1 NaOH for neutralization with phenolphthalein. After titration and sterilization the broth has a *PH of 7.1 to 7.3.*

In contains naturally from 0.2 to 0.10 mg. of iron per 100 c.c.

Experiments were conducted to determine if the addition of iron salts exerted a favorable influence upon the growth.

Conclusions:

1. Glycerin-broth to which 20 mg. per 100 c.c. of ferric sulphate were added averaged 41 per cent heavier pellicle than broth without iron. Growth, however, is slower upon the iron broth during the first few weeks.
2. Growth is favorably influenced by *ferric sulphate in media ranging in PH from 5.9* to 8-plus.

3. The favorable influence is due to the Fe ion and is not dependent upon the kind of iron salt added, ferric citrate, ferric sulphate, and ferric glycerophosphate all producing comparable effects when used in the proportion indicated above.

4. The most favorable concentration of iron appeared to be from 0.01 to 0.3 gm. of ferric sulphate per 100 c.c.

The rate of growth upon iron broth is at first lessened but in the later weeks the growth is more rapid and heavier than upon the control media.

The effect of increasing acidity and consequent change in the PH of the medium is less marked in broth containing iron, and the addition of iron in small amounts to glycerin-broth is therefore advantageous in the culture of B. tuberculosis.

Malone, R. H.: A Method of Estimating the Degree of Solubility of Microorganisms in Bile and "Bile Salt" Solutions. Indian Jour. Med. Res., April, 1923, x, 1144.

The method described by Malone for determining the degree of bile solubility of bacteria is based upon the principle that the opacity of a given bacterial suspension is directly proportional to the weight of the bacteria per unit volume of suspension.

The bacterial suspension is made from cultures grown on 5 per cent sheep blood agar slants for 20 to 24 hours at 37° C. The growth is suspended in normal saline, shaken thoroughly and filtered to remove clumps.

The removal of clumps may be neatly accomplished by twisting a small piece of sterile cotton around the end of a capillary pipette as in making a swab. This acts as an effective filter when the bacterial suspension is sucked into the pipette. The cotton should be removed before the suspension is blown out.

The clump-free suspension thus obtained is standardized against opacity tubes such as are used in the nephelometric standardization of vaccines. An acceptable concentration is approximately 2,800 million per centimeter of suspension (1.72 mg. of dry bacterial substance).

The solvent is prepared by dissolving one gram of sodium taurocholate in 1 c.c. of warm normal saline. One volume of this solution is added to nine volumes of bacterial suspension.

A set of standard opacity tubes, a capillary pipette of 1.5 to 2 mm. bore, internally lined and coated at the tip with paraffin, and small test tubes of the same bore as the opacity tubes (7 cm. long and about 7 mm. bore), are further required.

Method.—

1. Place one volume of sodium taurocholate solution in one of the small test tubes, using the capillary pipette.
2. Add nine volumes of bacterial suspension and mix.
3. Quickly determine the opacity by comparison with the standard tubes.
4. Incubate in a 37° C. water-bath for 30 minutes.
5. Remove from the bath, mix, and again read the opacity.

Tests should be made in duplicate and the readings, which should not vary more than one tube, are made to the nearest tube. Control tubes of the bacterial suspension are not required.

The test is thus recorded:

Opacity of suspension	10
Opacity after addition of bile solution	8
Opacity after incubation	2

The degree of solubility is, hence, recorded as 6.

Capuani, G.: Alizarin Test in the Diagnosis of Tuberculosis. Riforma med., April 13, 1925, xli, 345.

A known nontuberculous sputum is used as a control and diluted in a test tube in the proportion of one to five with distilled water.

A similar dilution of the specimen to be tested is made in another tube. To each

sample are added 20 drops of a 1 per cent alcoholic solution of alizarin. The tubes are stoppered and vigorously shaken and read after 20 minutes.

In tuberculous sputum a purplish precipitate occurs, the supernatant fluid being red.

In nontuberculous sputum, the entire tube is purplish red and becomes transparent within a few hours, and, if a precipitate forms, it is bluish and nubecular.

In Capuani's investigations the test proved neither specific nor conclusive, and he believes it to be of little value.

Kessel, J. F., and Willner, O.: Some Clinical and Laboratory Aspects of Amebiasis with a Preliminary Report of Yatren Treatment. China Med. Jour., May, 1925, xxxix, No. 5, p. 383.

The only certain method of laboratory diagnosis is by microscopic examination, and stools should be examined on six consecutive days before a negative report can be given.

A preliminary examination may be made by examining a smear in Donaldson's iodine-eosin stain: 5 per cent aqueous solution of potassium iodide saturated with iodine and mixed with an equal volume of saturated aqueous eosin.

The best method is to fix the smears in Schaudin's fluid for two minutes at 60° C. The formula for Schaudin's fluid is:

Sat. aqueous solution of mercuric chloride	200 c.c.
Alcohol, 95 per cent	100 c.c.
Glacial acetic acid	15 c.c.

For the best results a special laboratory department should be created where the number of such specimens is large.

Very favorable results were secured with yatren treatment, which the authors believe will be a satisfactory therapeutic agent in both the acute and chronic forms of amebiasis.

Jennings, J. E.: The Gram-Positive Anaerobes in Appendicitis and Its Complications. New York State Jour. Med., January 16, 1925, xxv, No. 1, p. 1.

Jennings believes that the greater number of patients dying of peritonitis secondary to appendicitis die of an intoxication, and that the clinical manifestation of this intoxication is not unlike the classical descriptions of gas gangrene.

He reviews the literature relative to the presence of the gas bacillus in appendicitis, especially when associated with gangrene and reports his own studies in which, in gangrenous appendicitis, the Welch bacillus was frequently found both in the appendix and the peritoneal fluid.

Suspicious material is emulsified in sterile broth. A portion is inoculated into anaerobic milk tubes and another portion is injected into both lobes of the liver of a guinea pig, a slight laceration being produced by twisting the needle at the time.

The animal is then killed, placed in the incubator for two hours, and then autopsied.

The organisms may be demonstrated in smears and cultures from the liver, heart blood, and peritoneal exudate.

Saxl, P., and Kelen, A.: The Trypsin-Flocculation Reaction in the Serum and Its Biological Significance. Wien. Arch. f. inn. Med., November, 1925, No. 11, p. 549.

One gram of trypsin (Merck) is dissolved in 100 c.c. of normal saline containing 0.5 per cent tricresol; is allowed to stand one or two hours at room temperature, and is finally cleared by filtration. The tryptic activity, as tested by the digestion of casein, remains stable for two weeks if the solution is kept in the refrigerator.

Blood is collected before breakfast, and the serum is allowed to stand in the refrigerator for 24 hours.

A series of serum dilutions from 1:25 to 1:52,000 is set up in small (Wassermann) tubes. The last tube of the series is the trypsin control and contains only saline. To the first serum tube 1 c.c. of the 0.5 per cent tricresol-saline is added; to all the others 1 c.c. of trypsin-saline-tricresol solution.

All tubes are then incubated 24 hours at 37° C.

The serum control (Tube 1) is clear or slightly turbid; the trypsin control is clear, and the other tubes are either flocculated or distinctly turbid.

Normal serum always flocculates the trypsin solution. The reaction seems to be related to the trypsin content of the intestinal canal, decreasing and increasing with it.

It is suggested that the reaction may be of value in pancreatic conditions.

Montenegro, J.: Cutaneous Reaction in Leishmaniosis. Arch. Dermat. and Syph., February, 1926, xiii, No. 2, p. 187.

The growth of leishmania after 15 to 20 days on Nicolle's medium is washed off with normal saline. The suspension is twice washed with normal saline and recovered by centrifuging. Coca's fluid is added to the sediment from the last washing in sufficient amount to make a turbid suspension.

The liquid is then covered with toluol and allowed to stand at room temperature for three days. The container should be shaken daily to facilitate maceration.

The suspension is then centrifuged and the clear liquid—which is the extract used in the test—is pipetted off.

Before using it is cultured for sterility in gelatine at 37° C. and on Nicolle's medium at room temperature.

A sufficient quantity is injected intradermally to produce a wheal 8 mm. in diameter. This is usually about 0.2 c.c.

In positive cases redness, edema, and papule formation occur in 24 hours.

The alkaline Coca's fluid is injected as a control.

In 37 cases positive reactions were obtained in 32. No reactions were seen in 33 controls. Reactions were not obtained in cured cases.

Prince, L. H.: A Rapid Stain for Nerve Tissue. Bull. XI, Internat. Assn. Med. Museums, May, 1925.

Stain:

Fuchsin f. bac. Gruber, sat. aq. sol.	3– 5 gtt.
Erythrosin Gruber 1 per cent aq. sol.	15 gtt.
Methyl orange, Campbell and Bell, sat. aq. sol.	40 gtt.
Aniline blue porous, Campbell and Bell, sat. aq. sol.	25 gtt.

Mix in the order listed and let stand 24 hours with frequent agitation during this period. No precipitates are formed and the solution appears to be stable so that multiples of the unit may be prepared if desired.

Investigations are now in progress to perfect this combination with American dyes.

Tissues are fixed in formalin and cut in paraffin.

Sections are passed through xylol, 95 per cent alcohol and water to prepare for staining.

The excess of water is drained off and the slide covered with the stain for 10 to 30 seconds. It is then washed in running water, dehydrated in absolute alcohol (copper sulphate-dehydration is satisfactory), blotted carefully but quickly, cleared in xylol, and mounted in balsam.

Several sections should be stained simultaneously and removed at intervals of 5 to 60 seconds in order that the reactions of various elements to the stain may be noted.

Staining reactions:

Nerve cells in general: light to dark purple with deeply stained Nissl bodies.

Normal cells: nuclei blue: nucleolus orange-red.

Usually deeply stained in 20 seconds.

Myelin: pink to deep red, especially if this substance is granular. The axis cylinder is almost black, tending to orange as disintegration progresses.

Neurolemma: light or dark blue.

Neuroglia: blue.

Erythrocytes: orange to red orange.

Lymphocytes: pale blue.

Corpora amylacea: pale blue.

After the section has been taken from the first alcohol it may be treated for 10 to 20 seconds with 1 per cent glacial acetic acid in 90 per cent alcohol. The stain is then applied without previous washing of the section in water.

Marked intensity in staining of some of the elements is secured, especially in neuroglia and connective tissues and myeln. Nerve-cell detail, however, may be obscured if the staining is too long.

Sheridan, W. F.: A Rapid Hematoxylin-Eosin Staining Method for Paraffin Sections. Bull. XI, Internat. Assn. Med. Museums, May, 1925.

Harris' hematoxylin stain is used and staining may be completed in five minutes.

Sections should not be over 5 micra in thickness and should be mounted with albumin.

Method.—

1. Xylol 2 minutes.
2. Rinse with 95 per cent alcohol (from drop bottle).
3. Hematoxylin, Harris, 2 minutes.
4. Rinse with 95 per cent alcohol.
5. Acid alcohol (1 per cent HCl in 70 per cent alcohol), 15 or more seconds.
6. Rinse with 95 per cent alcohol.
7. Ammoniated alcohol until rose color is replaced by blue: about one minute (freshly prepared before using: stronger ammonia 4 drops, alcohol 95 per cent 50 c.c.).
8. Rinse with 95 per cent alcohol.
9. Eosin (0.25 per cent in alcohol 95 per cent) 30 seconds.
10. Rinse with acetone from drop bottle. Acetone should be water-free and should not give more than a faint turbidity with xylol
11. Xylol—clearing is instantaneous.
12. Mount in balsam.

Wearn, J. A., and Richards, A. N.: Quantitative Estimation of Minute Amounts of Urea. Jour. Biol. Chem., November, 1925, lxvi, No. 1, p. 275.

The method described was devised for the estimation of the urea content of glomerular urine in frogs and suffices to measure, with a probable error of 10 per cent, as little as 0.0005 mg. of nitrogen in the form of ammonia or urea.

The method consists in hydrolysis of the urea by heating with acid at 150° C. simultaneous nesslerization of the unknown and a known ammonium sulphate solution, and quantitative color comparison.

All reagents, of course, must be ammonia-free.

The tubes for color comparison were 8 cm. in length with an inside diameter of 5 mm. and were calibrated at 1 c.c. Readings were made by artificial light diffused through "daylight" glass, the tubes being held in a rectangular rubber mat to exclude all light not passing through the tubes

The method is described as tested on known solutions of urea and ammonium sulphate, the results obtained with glomerular urine being withheld until a sufficiently large series accumulates.

From twice recrystallized urea a solution containing 0.2 mg. N in 1 c.c. was prepared. A small amount (1 to 3 mg.) of this solution was drawn up in a capillary tube and discharged into a weighed capillary tube.

After weighing the tube and its contents, the fluid is transferred to a clean, dry, hard glass tube (pyrex) 6 inches long, inside diameter 5 mm., closed at one end and graduated at 1 c.c.

Enough normal HCl is added to make the concentration approximately 0.1 N. The upper end of the tube is sealed in the flame and tube autoclaved one hour at 150° C.

The top of the tube is then broken off and the inner surface of the top is rinsed with a few drops of water into the main bulk of the fluid in the larger fraction of the tube.

A series of comparative tubes are then charged with varying amounts of standard ammonium sulphate solution (1 c.c. equals 0.002 mg. N) the quantities chosen (0.1 to 0.3 c.c.) being such as to include the assumed nitrogen content of the specimen to be examined.

Normal HCl is then added to make the acid content exactly equal to that of the urea solution.

Then 0.2 c.c. of Nessler's solution is added to each standard tube and to the hydrolyzed urea solution. The contents of each tube are diluted to 1 c.c. and comparison made in the comparator.

With the method described the error was less than 10 per cent.

Maximon, A. A.: Role of the Nongranular Blood Leucocytes in the Formation of the Tubercle. Jour. Infect. Dis., November, 1925, xxxvii, No. 5, p. 418.

Maximon reports his studies upon the histogenesis of tubercle through the observation of tissue cultures inoculated with tubercle bacilli.

Rabbit leucocytes were obtained by centrifugation in a paraffined centrifuge tube and were coagulated by the addition of a few drops of embryonic extract. This coagulum was washed in Ringer's solution, cut into small pieces with sterile precautions and planted in dilute rabbit blood plasma and embryonic extract from 13- to 16-day rabbit embryos. Immediately after clotting of the plasma drop containing the explanted white blood layer, the edge of the fragment was inoculated with a culture of human tubercle bacilli grown on glycerol agar. The cultures were observed for seven days, being transplanted into fresh medium every second day. Observations were made of the living culture and after Zenker-formal and celloidin preparation of serial sections.

It was shown that the epitheloid and giant cells of tuberculous lesions have the same origin as the polyblasts or mononuclear exudate cells in common or purulent inflammation.

They arise partly from local fixed elements—histocytes, and partly from nongranulated *blood cells, lymphocytes and monocytes.*

The origin of epitheloid cells varies in accordance with the circumstances of the case, that is, the type of cells available. A sharp discrimination between monocytes and lymphocytes in their rôle in the formation of tubercle cannot be made. The monocytes respond more promptly and sooner reach the fully developed epitheloid stage, but the lymphocytes follow the same path.

The paper is illustrated with a color plate.

Pinner, M.: Complement-Fixation in Tuberculosis, III. Studies on the Nature of the Antigen. Am. Rev. Tuberc., October, 1925, xiii, No. 2, p. 142.

Pinner reports a study of complement-fixation in tuberculosis with more than 70 different antigen extracts prepared according to various methods which have been described.

Each antigen was tested, under identical conditions, against a mixture of 3 or 4 positive sera, and the antigenic quotient of each extract was determined by the method described by Kolmer.

He concludes that the main complement-fixing substances are closely associated with that fraction of the bacilli which is practically insoluble in ether, acetone and chloroform and soluble in alcohol. There is no satisfactory proof that the alcohol-soluble substances contain the only antigenic principle but no extract from which these substances have been removed furnishes a satisfactory antigen.

The degree of dispersion plays an important rôle in the antigenic value of lipoidal tuberculosis antigens.

The protein substances exert an inhibiting influence upon the lipoids in complement-fixation.

There is no evidence to indicate that certain tuberculous sera react only with protein and others only with lipoidal antigens.

Pinner, M.: Complement-Fixation in Tuberculosis, III. Studies on the Nature of the Antibody. Am. Rev. Tuberc., November, 1925, xii, No. 3, p. 233.

By some authors the complement-fixing bodies in tuberculosis are held to be associated with the globulin fraction, and by others, with the lipoidal fraction of the serum.

Pinner studies this question anew by digesting positive sera with trypsin and dialyzing them until chemically free from proteins and protein split products. Such preparations were found to contain complement-fixing bodies. They are not, therefore, globulins and must be either lipoids or proteins not broken up by trypsin. True liposes with a specific action on tubercle bacillus lipoids could not be found in sera containing complement-fixing bodies.

Complement-fixing bodies are readily destroyed by ether and any procedure including ether extraction is to be excluded from examinations for complement-fixing bodies in tuberculosis.

Pinner, M.: Complement-Fixation in Tuberculosis with Wassermann's Antigen. Am. Rev. Tuberc., April, 1925, xi, No. 2, p. 139.

In March, 1923, Wassermann introduced an antigen for tuberculosis complement-fixation tests, results of a trial of which are reported by Pinner.

Tubercle bacilli are extracted at incubator temperature with tetraline (a tetrahydrated naphthaline) in a shaking machine. After several weeks the bacterial residue is nonacid-fast. To this material lecithin is added. The preparation of the antigen is not further described, reference being made to Wassermann's original paper (Deutsch. med. Wehnschr., 1924, xlix, 303).

Using this antigen 646 patients were tested, 71.5 per cent of positive reactions being obtained.

A number of false positive reactions were obtained in nontuberculous patients and in pregnancy.

Pinner concludes that the test with Wassermann's antigen has limited diagnostic value and no prognostic significance.

Friedenwald, J., and Morgan, Z.: Observations on the Rosenthal Phenoltetrachlorphthalein Test as a Means of Determining Liver Function. Ann. Clin. Med., November, 1925, iv, No. 5, 415.

A report of results with this test in various conditions.

In catarrhal jaundice marked retention was noted, persisting for some time after disappearance of the icterus and thus suggesting a distinct disturbance of the liver cells.

In cirrhosis of the liver the findings were variable.

Definite retention was noted in all cases of hepatic cancer; in syphilis of the liver the degree of retention is related to the degree of involvement.

In affections of the gall bladder in which the liver is not involved, retention does not occur.

As an addendum, the authors report that, in their limited experience with it, the bromosulphthalein test is equally valuable and has many advantages.

Greene, H. M.: Lumbar Puncture and the Prevention of Postpuncture Headache. Jour. Am. Med. Assn., Feb. 6, 1926, lxxxvi, 391.

Greene is convinced that postpuncture headache is due to loss of fluid by leakage which eventually leaves the brain without a water cushion.

He uses, therefore, a needle of small caliber, the point of which is rounded, tapering, and sharp rather than blunt and cutting, thus avoiding any severance of the fibers of the spinal dural sac and reducing leakage to a minimum. These special needles were made by the Becton, Dickinson Co.

His further precautions are:

1. The patient is placed upon the table in a comfortable position and—this being an essential point—should remain motionless while the needle is in the dural sac.
2. A local anesthetic should be used in both the skin and deeper tissues.
3. A special needle as described, gauge 22, is used.
4. After fluid appears, the sample should be aspirated.

In 215 consecutive punctures so performed, headache occurred only in two, in many instances the puncture being done at noon and the patient returning to work without ill effect.

Berman, L.: The Diagnostic Criteria of Chronic Parathyroid Insufficiency with Special Reference to the Phosphate Content of the Blood. Am. Jour. Med. Sc., February, 1926, clxxi, No. 2, p. 245.

Berman thus tabulates the criteria of value in the diagnosis of this condition and as gauging the effect of treatment:

1. Dystrophies of the hair, nails, teeth and skin.
2. Mechanical hyperexcitability of the nerves: Trousseau and Chvostek phenomena.
3. Electrical hyperirritability of the peripheral nerves.
4. Diminished calcium content of the blood.
5. Decreased phosphate content of the urine and phosphate retention in the tissues with increased phosphate content of the blood.

In twenty cases the blood calcium was within the normal limit of 9-11 mg. per 100 c.c.

In all but one, however, there was a definite increase in the inorganic phosphate of the blood, one showing 6 mg., nine having over 5 mg., and the average amount being 4.89 mg. per 100 c.c. The blood calcium averaged 10.2 mg.

In 37 nonparathyroid controls the calcium averaged 10.9 mg. and the blood phosphates 3.96 mg. per 100 c.c.

Vigneaud, V., and Karr, W. G.: Carbohydrate Utilization. I, Rate of Disappearance of d-Glucose from the Blood. Jour. Biol. Chem., November, 1925, lxvi, No. 1, p. 281.

In view of the clinical significance attaching to the rate of disappearance of glucose from the blood by means of tolerance tests, the authors comment that but little is known of the mechanism involved and report studies upon various factors of possible influence.

Their studies were made upon rabbits.

Further evidence is offered that this mechanism is practically independent of the rate of absorption from the intestine.

Fasting invariably decreases the rate of disappearance of glucose following a hyperglycemia from ingestion of glucose.

After a period of fasting, specific foodstuffs vary the rate. Proteins 4 hours before or with the glucose test meal have little or no influence; 18 hours before, greatly decrease the rate. Fat given 18 hours before also decreases the rate of disappearance. Glucose given 18 hours before the glucose test meal slightly increases the rate.

Adrenalin 18 hours before greatly increases the rate of disappearance; morphine, although producing a hyperglycemia, produces no increase.

A glucose meal immediately after an insulin hypoglycemia has returned to normal shows a decreased rate of disappearance.

Sodium bicarbonate during a fast prevents the decrease but blood PH and CO_2 studies show that acidosis is not a predominant factor in causing this change during a fast.

These studies indicate the need of controlling the diet, period of fast, and medication before performing tolerance tests.

Forster, E.: The Spirochetes in Malaria-Treated Paralytics. München. med. Wchnschr., Dec. 18, 1925, li, 2197.

Three patients were studied. Material withdrawn by brain puncture was examined by dark-field illumination. Spirochetes were found in two of the cases. In one they were very numerous and actively motile; in the second case only two spirochetes were found after prolonged search.

The material was then embedded and sectioned.

All three cases showed definite histologic changes.

In the patient in whom numerous organisms were found they were seen in the capillaries. The ganglia cells were markedly altered.

The report, though covering only three cases, is made because of previous reports that in malaria-treated cases spirochetes could not be demonstrated. It is suggested that this may be due to the uncertainty of staining methods for spirochetes.

Pucher, G. W., and Burd, L. A.: Note on the Stability of Dakin's Solution. Buffalo Gen. Hosp. Bull., April, 1925, iii, No. 1, p. 20.

The bleaching powder used in the preparation of Dakin's solution must contain at least 25 per cent available chlorine. The boric acid and sodium carbonate employed for PH adjustment must be free from organic impurities. For storage, dark, amber colored bottles must be used. These are washed clean from organic matter and are filled with Dakin's solution. After standing for 24 hours this solution is discarded and the bottles are filled with fresh diluted solution for use. If stored at 10 to 25° C. such solutions are stable for at least six weeks.

Knowles, F. C., and Decker, H. B.: Gastric Acidity in Acne Vulgaris. Arch. Dermat. and Syph., February, 1926, xiii, No. 2, p. 215.

There is a consensus of opinion that acne vulgaris is frequently accompanied by gastrointestinal disturbances; a study of the gastric acidity was made in 25 cases.

Ten cases showed a total acidity below 50, seven between 50 and 60, four between 60 and 70, and four over 70.

There was no relation between the acid values and the duration, type, or severity of the condition. When the acidity was low, improvement occurred under the administration of dilute HCl.

Cumming, W. M.: The Serology of the Bovine Type of the Tubercle Bacillus. Tubercle, London, December, 1925, vii, No. 105, p. 8.

The investigation herewith reported was undertaken to verify the contention that human and bovine tubercle bacilli are serologically identical.

Antihuman tubercle serum was obtained by the immunization of a rabbit with an emulsion of ether-acetone extracted human tubercle bacilli from a broth culture. The titer was 1:1600.

More difficulty was encountered in preparing an antiserum with a similar preparation of bovine bacilli, but one was finally secured with a titer of 1:800.

Emulsions were prepared from cultures on Dorset's or Lubenau's egg medium, extracted with acetone and ether, dried, ground to a paste with 1:4 sodium bicarbonate, and suspended in normal saline with 0.5 per cent phenol, the suspensions being standardized to contain 32,000 million organisms per centimeter. One c.c. of this suspension and 1 c.c. of antiserum in a strength equal to 16 times the titer were mixed and allowed to stand overnight at room temperature

After centrifugation, the supernatant fluid containing the antiserum in eight times the titer, was distributed into three sets of four tubes: into the first set was distributed 0.25 c.c. of unabsorbed serum so diluted that strengths equal to 8, 4, 3, and 2 times the titer were obtained, and 0.25 c.c. of a 2000 million per c.c. bacillary suspension. The final serum concentrations were thus 4, 2, 1.5 and 1 times the titer, acting upon 500 million organisms.

Similar volumes and dilutions of absorbed serum and 0.25 c.c. of a 2000 million per c.c. suspension of homologous organisms were added to the second set, and 0.25 c.c. of a 2000 million per c.c. suspension of bacilli to be tested was added to the third set.

In addition, 0.25 c.c. of the suspension of test organisms was set up against 0.25 c.c. of 1:12.5 dilution of normal rabbit serum.

The tubes were then incubated 24 hours at 56° C. in an oven or water-bath.

The experiments showed that tubercle bacilli of human and bovine types are not distinguishable by agglutination or absorption of agglutinins.

BOOK REVIEWS

(Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.)

*Organic Derivatives of Antimony**

THIS volume is one of the American Chemical Society's Series Service of Scientific and Technologic Monographs. Some twenty-five or more of these have already been published. It is hoped that "two rather distinct purposes may be served by these monographs. The first purpose, whose fulfillment will probably render to chemists in general the most important service, is to present the knowledge available upon the chosen topic in a readable form, intelligible to those whose activities may be along a wholly different line. Many chemists fail to realize how closely their investigations may be connected with other work which on the surface appears far afield from their own. These monographs will enable such men to form closer contact with the work of chemists in other lines of research. The second purpose is to promote research in the branch of science covered by the monograph, by furnishing a well digested survey of the progress already made in that field and by pointing out directions in which investigation needs to be extended."

The preparation of the present monograph was undertaken in connection with a study of antimonials which is being made in the Departments of Pharmacology and of Tropical Medicine of the Harvard Medical School.

The chemistry of organometallic compounds has been expanding very rapidly during the last few decades, owing largely to the marked physiologic activity of these substances. In this great class of compounds there are, on the one hand, some of the most powerful of the war gases and, on the other hand, some drugs which are indispensable in modern therapeutics. Although the organic arsenicals have been investigated much more thoroughly than the antimonials, studies in the latter field are being carried on quite actively in connection with researches on protozoal diseases because it appears that in certain of these diseases the antimonials are much more efficacious than the arsenicals.

The author has attempted to collect all the available literature on the subject and to present it in such a way that it will be useful both for those desiring a general knowledge of the organic derivatives of antimony and for those embarking on researches in this field. To this end the material has been so arranged that the first chapters are devoted to general discussions without the introduction of detailed directions for the production of the various compounds. The latter type of detailed information for the laboratory production of the various compounds is presented in the last chapters.

*Organic Derivatives of Antimony. By Walter G. Christiansen, Instructor in the Harvard Medical School. Cloth. Pp. 230. The Chemical Catalogue Company, Inc., New York.

The book contains ten chapters of which the sixth, written by Dr. George C. Shattuck, deals rather briefly, but in an interesting manner, with the use of organic antimonials in trypanosomiases, the leishmanioses, the schistosomiases, granuloma inguinale and leprosy. It also takes up the action of antimonials in the body and the methods of treatment with antimonials.

The book is a valuable contribution and will be of much use to those carrying on special investigations in chemotherapeutics or in the field of organometallic compounds.

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EDITORIALS

The Measurement of the Efficiency of the Circulation

A SATISFACTORY and practical laboratory method for the estimation of the efficiency of the heart in carrying on the circulation has not as yet been perfected. There is great need for such a procedure. The method of Henderson and Haggard,[1] using ethyl iodide, gives promise of being satisfactory. The principle is that the rate of absorption of a relatively slightly soluble gas, such as ethyl-iodide vapor, from the alveolar air into the blood is nearly proportional to the circulation through the lungs. The technic of the procedure is claimed to be simple, accurate, and reliable. The patient is made to inspire dilute ethyl-iodide vapor for ten minutes through a specially devised apparatus. Air samples are taken to determine: (*1*) the volume of air breathed per minute; (*2*) the concentration of ethyl iodide in the inspired air; (*3*) the concentration in the expired air; (*4*) the concentration in the alveolar air.

(*A*) The amount of ethyl iodide absorbed per minute is determined by multiplying factor *1* by the difference between *2* and *3*. (*B*) The amount of

ethyl iodide in the arterial blood is equal to the factor *A* multiplied by the *coefficient of solubility* of the gas (approximately 2.0). Then the volume of the blood flow through the lungs is determined by dividing *A* by *B*. The complete determination of the respiratory exchange, with oxygen consumption as a basis for indirect calorimetry, that is, the basal metabolism, is made simultaneously. One operator can carry out the procedure, including the determination of the minute amounts of ethyl-iodide vapor, by the accurate iodine-pentoxide method and also the analyses for oxygen and carbon dioxide in considerably less than an hour. The ethyl-iodide vapor has a conveniently *low air blood distribution coefficient, approximately 2.0, at body temperature.* The ethyl iodide is furthermore completely decomposed in the tissues so that the amount returning to the lungs in the venous blood is practically negligible. Further advantages claimed for the method are that it is applicable to practically all conditions and requires practically no cooperation upon the part of the patient other than to breathe as naturally as possible through a mouthpiece and valves for ten minutes.

J. W. Moore, using this method with a modification of apparatus so perfected that he could measure foot-pound work with the patient lying in bed and after exercise, had hoped to be able to determine even the extent of the lowering of the circulation efficiency in patients with heart disease and establish the limit of activity that a patient might safely engage in. He recently reported, however, only disappointment and apparent failure of the method at the American Medical Association meeting in Dallas. The method still deserves consideration and further attempts to apply it chemically.

Burwell and Robinson[2] have devised a more elaborate method based upon the principle of Fick. Calculation of the cardiac output by Fick's principle requires an accurate estimation of: (*1*) the amount of oxygen absorbed by the body per minute, and (*2*) the amount of oxygen taken up by each unit of blood as it passes through the lungs. Division of the total oxygen per minute by the oxygen taken up by each unit of blood will express the number of units of blood passing through the lungs each minute. The determination of the oxygen taken up by each unit of blood requires *accurate estimation of:* (*1*) the oxygen content of the blood as it leaves the lungs (arterial blood), and (*2*) the oxygen content of the blood as it enters the lungs (mixed, venous blood). The corresponding figures for carbon dioxide would serve as well in the application of *Fick's principle*. The *gas content* of the mixed venous blood can be approached only indirectly in man.

By a respiratory method, properly diluting the oxygen of the lung air by inspiring a 0.7 to 2 per cent oxygen mixture before each rebreathing, the gas mixture (O_2 and CO_2) in the rebreathing bag may be promptly brought into equilibrium with that of the mixed venous blood entering the lungs. Thus the lungs are used as an aeronometer. A tonometer is filled directly with this rebreathed *gas mixture and peripheral* venous blood of the subject is equilibrated with it under standard conditions. The peripheral venous blood so treated is, as it were, "artificial," mixed, venous blood, and the analysis of this for O_2 and CO_2 by the method of Van Slyke and Stadie, sup-

plies the desired data. The measurement of the output of the heart from this data, obtained by the application of the method described based upon Fick's principle, is accomplished as follows: $M = \frac{O}{W} \times 100$. M = minute output of the heart in cubic centimeters. O = c.c. of oxygen absorbed per minute. W = volumes per cent of oxygen utilized. As the gases of the blood are now commonly calculated as volumes of gas per 100 c.c. of blood, the formula can be conveniently employed.

The established normal for adults at complete rest varied from 3,500 c.c. per minute and 58 c.c. per beat to 6,800 c.c. per minute and 103 c.c. per beat. These figures lend support to the view that the output of the heart per beat is not fixed but variable. Further observations bearing upon this are anxiously awaited. The method, though complicated, is the most promising that has thus far been put forth. It may lead to a simpler clinical laboratory method of this most important quantitative function of the body, the efficiency of the cardiorespiratory system.

The clinical methods of estimating cardiac function are still quite crude, but standardized by some such exact method and elaborated by electrocardiographic and roentgenographic studies, the ordinary exercise tolerance tests may become increasingly valuable to the clinician as a means of establishing rational prognosis.

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—*G. R. H.*

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SUBJECT INDEX

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VALUABLE SUGGESTIONS FOR CONTRIBUTORS TO THE JOURNAL OF LABORATORY AND CLINICAL MEDICINE

"The four rules for the preparation of an article will then be: (1) Have something to say; (2) Say it; (3) Stop as soon as you have said it; (4) Give the paper a proper *title*."[1]

Let your phraseology express one meaning and one only. Be clear.[2]

Manuscript.—Manuscripts should be typewritten, with wide margins, and double spaced, on one side of paper 8½ by 11 inches in size. The original copy should be sent to the "Journal" and the carbon copy retained by the author. Number the leaves consecutively, beginning with the title page. Put your name and address on the manuscript.

Illustrations.—Illustrations should be clear, preferably pen-and-ink drawings. Of photographs send a good print rather than a negative. Have lettering parallel to the bottom and top margins, and of sufficient size to be clear if cut is to be reduced. Tracings should be in black-and-white; avoid colors. Write your name on back of each picture; number them in one series (Fig. 1, etc.) to the end, and indicate in margin of the manuscript about where each is to be printed. See that the text references and "figures" correspond. Legends for illustrations should be written on a separate sheet.[3]

Bibliographic References.—Give only references actually consulted. If an article is known only through an abstract give reference to the abstract in addition to that of the source. References are printed to be of help in further reading; therefore they must be complete, concise, and correct. Follow the style of the "Index Medicus" and "Index-Catalog of the Library of the Surgeon-General's Office." Be conservative in the use of abbreviations.[4]

Arrangement.—As authors are quoted in the text give each a number in the order of citation, and number the bibliographic reference with the same number. Arrange the references in a list at the end of the article in the order of the numbers (see below), or arrange items in alphabetical order according to last names of authors, and distinguish between articles by the same author by the use of the date after his name in the text.

Foot-notes.—Where an author wishes to use foot-notes at bottom of each page instead of the bibliography at end of article, the foot-notes should be written in the text, but separated from it by horizontal lines above and below, or *better*, place them at bottom of each page. Use figures to indicate these foot-notes, and number consecutively (1, 2, 3, etc.) throughout the article. If in addition to the bibliography mentioned above it is desired to use foot-notes on certain pages, these can be indicated by an asterisk (*).

Final Reading.—Let some one other than the author read the manuscript with these directions in mind.

Shipment.—Send manuscript flat, postage paid, to the editor, Dr. Warren T. Vaughan, Medical Arts Bldg., Richmond, Va.

Proof-reading.—Read carefully, with special attention to spelling of names and bibliographic data. Make corrections *in the margin* only with lines drawn from the revision to the point of change in the text. Answer queries in the proof by making correction or crossing out the query. Verify your references from the sources, not from your carbon copy.

References. (Read these.)

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